THE LIPOTROPIC EFFECT OF VITAMIN B_{12} IN THE DOG [1]

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ONE FIGURE

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INTRODUCTION

During the course of some studies on acetaldehyde metabolism in this laboratory, a defect of liver function was produced in adult dogs maintained on a synthetic ration (Westerfeld, McKibbin, Roemmelt and Hilfinger, '49). The dogs lost no weight and presented no other signs of nutritional deficiency. A succeeding study using 5 weanling puppies (unpublished data) resulted in poor growth of the animals and deaths after 4 to 5 months on ration, with liver fat often exceeding 50% lipid dry weight. We did not suspect a deficiency of known lipotropic factors, although there is limited information on the choline requirement of dogs on rations similar to ours (McKibbin, Thayer and Stare, '44). Interrelationships with fat and methionine make it difficult to define choline needs except in terms of a particular ration. Furthermore, recent investigations reported by Schaefer and associates (Schaefer et al., '49, '50a, b) and others (Gyorgy and Rose, '50; Stekol, Bennett, Weiss, Halpern and Weiss, '50; Stokstad, Jukes,

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Brockman, Pierce and Broquist, '50; Gillis and Norris, '49) have indicated a relationship between vitamin B_{12}, folic acid and the requirement for methylating substances. It is the purpose of this paper to report a deficiency of a lipotropic substance in a purified ration utilizing several different sources of carbohydrate (Elvehjem and Krehl, '47) and to identify this substance with vitamin B_{12}.

TABLE 1

Composition of rations

CONSTITUENTS	DIET					VITAMIN SUPPLEMENTS ADDED TO EACH RATION	
	I	II	III	IV	V		
	gm/100 gm ration					*mg/100 gm ration*	
Vitamin-test casein [1]	19	19	19	19	19	Thiamine hydrochloride	0.2
Sucrose	28	68				Riboflavin	0.4
Glucose	40		68			Pyridoxine hydrochloride	0.2
Beta-lactose				68		Calcium pantothenate	0.5
Starch					68	Choline chloride	100.0
						Nicotinic acid	2.5
Cottonseed oil	7	7	7	7	7	Folic acid	1.0
						Biotin	0.2
						Inositol	400.0
Salts [2]	4	4	4	4	4	Menadione	1.0
Cod liver oil	2	2	2	2	2	34% mixed tocopherols	10.0

[1] General Biochemicals, Inc.

[2] P. H. Phillips and E. B. Hart. J. Biol. Chem., *109:* 657, 1935. An additional 2.8 gm of manganese sulfate were added per kilogram of salt mixture.

EXPERIMENTAL AND RESULTS

A. Effect of carbohydrates on the production of the deficiency

Three litters of weanling puppies (18 animals) were dewormed, actively immunized against distemper, and apportioned on diets I to V (table 1). One puppy from each of two litters remained on the stock diet to which meat was added, and these animals served as controls. Ad libitum feeding was maintained throughout.

Plasma alkaline phosphatase determinations (Bodansky, '33) were used as indices of liver function. Values in excess of 8 to 10 units were considered abnormal. Terminal total liver lipid analyses (McKibbin, Thayer and Stare, '44) were made and are reported as per cent lipid dry weight. Values up to 20% were considered within the normal range.

TABLE 2

Effect of different carbohydrates on growth and total liver lipids

DOG NO.	RATION	DAYS ON RATION	AVERAGE OVER-ALL WEIGHT GAIN FOR FIRST 10 WEEKS (% INITIAL BODY WEIGHT/ WEEK)	AVERAGE GAIN FOR EACH GROUP (% INITIAL BODY WEIGHT/ WEEK)	TERMINAL LIVER FAT (% $CHCl_3$ EXTRACT)	TERMINAL PLASMA ALKALINE PHOSPHATASE (BODANSKY UNITS) PER 100 ML
25	Control	90	20.1	20.7	13.7	4.01
29		97	21.3		6.5	3.43
20	Glucose-sucrose (diet I)	126	11.8	13.7	15.0	9.55
30		90	15.6		19.0	...
22		46	died at 6 weeks		83.6	...
26	Sucrose	90	19.7		9.8	12.78
31	(diet II)	108	14.4	17.55	46.9	17.45
32		104	21.6		36.2	19.94
33		104	14.6		23.7	....
23	Lactose	146	8.3		15.0	6.44
35	(diet III)	89	11.5	10.7	18.0	21.2
36		80	10.3		18.0	34.46
28	Glucose	42	died at 6 weeks		76.0	....
34	(diet IV)	97	16.3	13.85	23.5	17.74
21		126	11.4		5.5	3.51
24	Starch	123	12.5		4.9	6.44
37	(diet V)	100	15.4	14.7	29.1	...
38		104	16.2		13.9	...

The experimental data are summarized in table 2. The control group attained weight gains superior to those of animals on any of the synthetic rations. Thus puppies on dog chow, milk and meat made average over-all gains equivalent to 20.7% of initial body weight per week for the first 10

weeks, while the gains of those on purified rations ranged from 10.7% to 17.5%. Puppies of this age may be expected to gain from 15% to 30% of their initial body weight per week (McKibbin et al., '44, '45; Arnold and Elvehjem, '39).

Seven of the 16 animals receiving the synthetic rations developed fatty livers. The several carbohydrates utilized did not greatly affect the onset of the deficiency. Four fatty livers appeared on the sucrose ration, one on the starch ration, and two on the ration employing glucose as the carbohydrate. The normal liver fat analyses for animals receiving lactose may have been coupled with grossly poor growth, but it is possible that lactose per se may exert a lipotropic action (Artom and Fishman, '47).

The terminal plasma alkaline phospatase values were generally correlated with the degree of fatty infiltration. High values also occurred in the lactose-fed animals in which there was no fatty infiltration of the liver. These elevations may, however, have been due to an altered metabolism of calcium and phosphorus (Handler, '47).

B. Effect of lipotropic factors

Two additional litters, totalling 13 weanling puppies, were treated as previously described and were placed on ration at approximately 8 weeks of age. The glucose-sucrose ration (diet I) was used with either 100 mg or 75 mg of choline per 100 gm of ration.

Weight gain on this ration was again suboptimum in both litters. The average over-all gain for the first 10 weeks of the experiment was only 6.2% of the initial body weight per week for both levels of choline. However, only 4 of the animals developed abnormalities of liver function as measured by plasma alkaline phosphatase, total cholesterol and bromsulfalein rentention (McKibbin, Thayer and Stare, '44). Two of these puppies received 75 mg % choline; two had been given 100 mg % choline.

TABLE 3

Effect of vitamin B_{12} and choline on liver function

DOG NO.	INITIAL RATION Diet I	DAYS ON RATION	BODY WEIGHT	TOTAL PLASMA CHOLESTEROL	PLASMA ALKALINE PHOSPHATASE	BROMSULFALEIN RETENTION	PLASMA LIPID PHOSPHORUS	TERMINAL LIVER FAT IN % $CHCl_3$ EXTRACT
			kg	*mg/100 ml*	*Bodansky units per 100 ml*	*µg/ml*	*micromoles per 100 ml*	
43	75 mg % choline	156	6.9	142	36.1	45.5		
		167	7.2	167	28.7	47.0		
		180	7.15	150	27.2	47.2		
		182	...	approximately 1 gm choline daily by capsule and on ration				
		191	7.2	222	14.2	48.6		
		210	7.3	250	10.2	...		
		241	7.13	253	8.18	...	573	
		252	7.16	243	11.0	58.8	496	
		255	...	discontinued choline — subq. injection 400 µg vitamin B_{12}[1] on alternate days				
		268	7.17	256	10.5	46.0	493	
		283	6.9	254	9.48	40.0	549	
		284	...	sacrificed				11.5%
44	75 mg % choline	162	9.77	181	24.8	50		
		224	9.75	170	23.4	66		
		240	9.55	167	17.0	63	452	
		251	9.66	210	17.3	61	448	
		256	9.4	started inject. 400 µg vitamin B_{12} on alternate days				
		267	9.25	220	9.0	47.4	451	
		282	9.39	227	6.1	48.0	435	
		286		sacrificed				9.47%
49	100 mg % choline	156	6.35	172	18.7	24		
		167	6.6	190	19.2	30		
		180	6.6	180	19.1	32		
		182	..	started injection 10 µg vitamin B_{12} twice weekly				
		191	6.6	257	11.5	33.4		
		200	6.62	...	9.76	39.0		
		210	6.6	225	8.86	...		
		286	6.84	sacrificed				9.86%
50	100 mg % choline	116	7.41	182	11.9	...		
		149	7.9	160	13.3	41		
		156	7.57	187	13.7	40		
		158		subq. injection 10 µg vitamin B_{12} twice weekly				
		166	8.12	210	6.58	42.5		
		179	7.95	210	6.96	45.0		
		181		discontinued vit. B_{12} — approx. 1 gm choline daily by capsule and on ration				
		190	8.15	215	5.84	38.6		
		199	8.25	200	5.58	59.0		
		209	8.34	210	5.54	40		
		273	8.52	sacrificed				9.13%

[1] Vitamin B_{12} for these studies was "Cobione" furnished by Merck and Co., Rahway, N. J.

The treatment of the 4 animals developing defects in liver function was as follows: no. 43 was given supplementary choline followed by vitamin B_{12}; no. 44 and no. 49 were treated with vitamin B_{12} alone; no. 50 was given vitamin B_{12} first, followed by choline. The effects of these supplements on liver function are summarized in table 3. It is apparent that both choline and vitamin B_{12} reduced the plasma alkaline phosphatase in all 4 animals. Total cholesterol was increased in

TABLE 4

Effect of vitamin B_{12} on liver function in dogs given a choline-free diet

DOG NO.	INITIAL RATION	DAYS ON RATION	BODY WEIGHT	TOTAL PLASMA CHOLESTEROL	PLASMA ALKALINE PHOSPHATASE	BROMSULFALEIN RETENTION	PLASMA LIPID PHOSPHORUS	TERMINAL LIVER FAT IN % $CHCl_3$ EXTRACT
			kg	*mg/100 ml*	*Bodansky units per 100 ml*	*μg/ml*	*micromoles per 100 ml*	
46	75 mg %	288	13.33	224	6.42	51.5	510	
	choline	289		begun on choline-free diet				
		309	13.37	214	10.3	84.7	400	
		329	12.95	163	15.8	...	360	
		371	11.37	70	24.5	92	153.6	
		384	10.54	35.4	21.6	121.2	76.5	
		385		liver biopsy				67.6%
		385		started injection 400 μg vitamin B_{12} on alternate days				
		399	9.78	115	17.8	47.8	302	
		406	9.85	154	11.3	...	362	
		414	10.32	169	8.38	36.0	394	
		416	10.5	sacrificed				25.95%
52	75 mg %	119	7.72	137	8.79			
	choline	163	8.43		9.36	22.4		
		174	8.75	begun on choline-free diet				
		195	8.45	90	29.3	38.0		
		224	8.38	65	37.0	47.0		
		241	8.11	65	35.5	63.5	182	
		244		liver biopsy				24.6%
		244		started injection 400 μg vitamin B_{12} on alternate days				
		252	7.67	137	25.5	36.4	393	
		257	7.91	146	18.6	31.4	427	
		267	7.93	133	11.4	35.4	374	
		274	8.55	137	9.13		394	
		282	8.6	146	6.7	31.8	405	
		283	8.6	sacrificed				12.01%

dogs 43, 49 and 50. Bromsulfalein retention was essentially unchanged in all 4. It should be noted that only mild deficiencies existed in these 4 dogs.

Two of the remaining animals, dogs 46 and 52, were placed on a choline-free diet on the 289th and 174th experimental days, respectively (table 4). Prior to this time neither animal had shown indications of a liver defect other than some elevation in bromsulfalein retention. After three weeks without choline, both dogs developed distinct abnormalities in

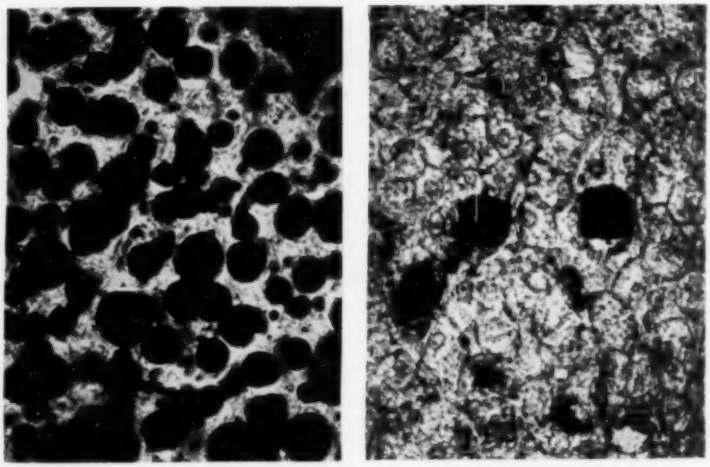

Fig. 1 Lipotropic effect of vitamin B_{12} on dog 46 fed a choline-deficient diet. Left, liver biopsy sample. Right, terminal liver section after treatment. Osmic acid. × 570.

liver function. This seemed to indicate a borderline nutritional status with respect to methylating substances. Plasma lipid phosphorus (McKibbin and Taylor, '49a) was used in addition to the other indices of liver function. A liver biopsy was taken from dog 46 after 96 days without choline and from dog 52 after 70 days. These demonstrated a severe and moderate degree, respectively, of fatty infiltration of the liver. Both dogs were given vitamin B_{12} immediately following the biopsy and were continued on this same choline-free ration.

A remarkable improvement in liver function occurred in both dogs during the 4-week period of treatment, as is shown in table 4. The dogs were then sacrificed and the livers examined histologically and analyzed for total lipids. A notable decrease in liver lipids was apparent (fig. 1, table 4).

TABLE 5

Effect of vitamin B_{12} and choline on the composition of the liver lipids

(All values are given in micromoles per gram of dry lipid-free tissue)

DOG NO.	TREATMENT	TOTAL LIPID NITROGEN	TOTAL LIPID PHOSPHORUS	TOTAL LIPID CHOLINE NITROGEN		TOTAL LIPID SPHINGOSINE NITROGEN	
					Per cent of total lipid nitrogen		Per cent of total lipid nitrogen
46	None (biopsy)	162.5	168.8	66.0	39.1	24.25	13.4
46	Vit. B_{12}	131.7	138.0	65.9	47.8	11.78	8.53
52	Vit. B_{12}	138.2	146.0	67.2	46.0	16.06	11.0
44	Vit. B_{12}	111.3	124.9	64.6	51.8	12.2	9.77
49	Vit. B_{12}	126.0	128.3	59.1	46.1	14.2	11.1
50	Vit. B_{12} then choline	116.8	117.4	60.5	51.5	12.9	11.0
43	Choline	136.4	142.8	79.3	55.5	15.38	10.8
54	No supplement [1]	152.3	169.6	61.3	36.2	15.57	9.18
45	No supplement [2]	107.6	109.9	43.8	40.7	11.43	10.6

[1] Dog 54 was given a choline-free diet from the 172nd day to the 360th day, when it was sacrificed.

[2] Dog 45 was given a choline-free diet from the 296th day to the 447th day, when it was sacrificed.

Since there is a characteristic change in the pattern of liver lipids in the choline-deficient dog (McKibbin and Taylor, '50), the livers of several of the animals of this series were analyzed for total lipid nitrogen, phosphorus, choline and sphingosine by methods previously reported (McKibbin and Taylor, '49a, b). These data are given in table 5 and show a reversal of the choline deficiency pattern resulting from the administration of vitamin B_{12}.

DISCUSSION

It seems clear from the foregoing results that a deficiency of vitamin B_{12} may occur in dogs on the type of ration used in our studies. The deficiency appears to manifest itself primarily in change in the metabolism of labile methyl groups, resulting in a liver disorder of varying severity in different litters of animals. Fatalities may be observed in 4 or 5 months, as experienced in the studies utilizing several carbohydrates. On the other hand, the deficiency may be a very mild one, detectable only with liver function tests and some impairment of growth. Routine hematocrit determinations on our animals revealed no marked blood changes associated with the deficiency. Ruegamer, Brickson, Torbet and Elvehjem ('48) have described a macrocytic anemia in niacin-deficient dogs on a 19% casein ration. Liver extract was necessary, in addition to niacin and folic acid, to cure the anemia.

The effect of vitamin B_{12} in curing the fatty infiltration of the liver in dogs 46 and 52 is particularly significant in that neither animal was receiving choline. Both animals responded to vitamin B_{12} therapy on a ration containing 19% casein and therefore 0.66% methionine as the only methylating substance. This response was evident in a decrease in total liver lipids as measured chemically and histologically, in improvement in liver function as measured by all the tests used, and finally by the change in the pattern of the liver lipids in dog 46 (table 5). The lipid pattern in the biopsy sample was characteristic of the choline-deficient puppy; that after vitamin B_{12} supplementation was typical of choline-fed control dogs (McKibbin and Taylor, '50). The alteration in the lipid pattern which occurred during treatment was shown in the higher percentage of lipid choline nitrogen and the decrease in the percentage of lipid sphingosine nitrogen. Although the nitrogen base pattern of the liver biopsy sample from dog 52 was not determined, the terminal liver sample showed a pattern typical of choline-fed control puppies.

Terminal liver lipid patterns of all dogs given either vitamin B_{12} therapy or supplementary choline were equally char-

acteristic of choline-fed control puppies. Data on dogs 45 and 54 are presented for purposes of comparison. These animals received a choline-free diet for several months before sacrifice and serve to illustrate the decrease in the per cent of choline N in the liver lipids characteristic of choline deficiency. The per cent of lipid sphingosine was not elevated in these animals. There was no relation between the absolute amount of lipid choline per gram of tissue and the supplements used. Total liver lipid nitrogen and phosphorus varied greatly. These relationships have been previously observed (McKibbin and Taylor, '50) in puppies.

The requirement of the dog for labile methyl donors must therefore be completely revised in terms of the vitamin B_{12} content of the ration. This fact has already been established with respect to the rat and chick by Schaefer et al. ('50a, b) and has been confirmed by others (Gyorgy and Rose, '50; Stekol et al., '50; Stokstad et al., '50; Gillis and Norris, '49).

Our data do not establish whether or not any methylating substances are necessary for the dog in the presence of an adequate supply of vitamin B_{12}. Our animals were all adult at the time of therapy. It is possible that both the growing and adult dog may be able to synthesize the entire requirement for labile methyl groups if given sufficient vitamin B_{12} and other factors necessary for this synthesis. Stekol et al. ('50) obtained growth with vitamin B_{12} in rats 30 days of age or older in the complete absence of methyl donors; however, Schaefer et al. ('50b) were unable to get full protection from renal damage in weanling rats on choline-free rations including vitamin B_{12} and folic acid.

It is surprising to have observed a vitamin B_{12} deficiency on our ration, since it is reasonably adequate in casein and low in fat. Few investigators report symptoms of a vitamin B_{12} deficiency on a comparable ration. Gyorgy and Rose ('50) observed a lipotropic effect of vitamin B_{12} in rats fed a ration containing 8% casein and 5% fat. This effect disappeared when the fat was elevated to 40%. Colby and Ensminger ('50) were unable to demonstrate significant improvement

from vitamin B_{12} in growth, hemoglobin levels or white blood cell counts in pigs given a 26% casein, 11% fat diet. Betheil and Lardy ('49) obtained a pronounced growth response from vitamin B_{12} in hyperthyroid rats fed a 22% casein, 5% fat ration. Anderson and Hogan ('50) produced this deficiency in young pigs using a purified ration containing 30% casein, but this ration contained 30% lard. It is possible that the dog may have a relatively high requirement for vitamin B_{12}; however, our limited data permit only the present inconclusive suggestions in view of the lack of comparable liver function studies in other species.

SUMMARY

1. A deficiency of vitamin B_{12} is reported in weanling puppies fed on a synthetic diet containing 19% purified casein and 9% fat. The deficiency varied in severity with different litters of animals and was evident in growth impairment, abnormalities of liver function and, in several instances, gross fatty infiltration of the liver.

2. Administration of either vitamin B_{12} or choline produced significant improvement in liver function in 4 dogs receiving the basal diet. Two dogs receiving a choline-deficient diet were cured of their fatty livers with vitamin B_{12} alone.

3. These findings suggest that the dog's requirement for methylating substances, like that of the rat and chick, is dependent upon the vitamin B_{12} content of the ration.

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STUDIES ON THE IODINE REQUIREMENTS OF WHITE RATS DURING GROWTH, PREGNANCY AND LACTATION[1]

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The iodine requirements of growing rats have been studied by several investigators. Levine et al. ('33b) determined these requirements using a modification of the rachitic ration of Steenbock and Black ('25). Employing as a criterion the prevention of a significant thyroid enlargement, the minimum iodine requirement with this ration was found to be approximately 1 to 2 μg per rat per day. Remington ('37) has pointed out that this diet was not ideal for the study of iodine deficiencies because the growth of young rats fed the diet was subnormal. He found that on addition of 2% dried pig liver to this diet, rats reached maturity and produced a normal number of viable young despite almost complete absence of iodine and colloid from the thyroid glands. No data were presented on the iodine requirements of rats during pregnancy and lactation. Halverson et al. ('49) reported that the iodine requirements of rats were slightly greater on a soybean-containing ration than on a basal ration similar to that used by Levine et al.

Since the use of feeds such as liver, soybeans, and cabbage, which have been reported to contain natural goitrogens, complicates the problem of determining iodine requirements,

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an attempt was made in the present work to prepare a ration suitable for iodine requirement studies during reproduction as well as growth without using any of the feeds which have been reported to contain goitrogens.

EXPERIMENTAL

Rations

The iodine content of numerous possible dietary components was determined by the combustion procedure described by Godfrey et al. ('51) and the catalytic method described by Chaney ('40). These analyses indicated that corn, wheat germ,[2] cottonseed oil, lean beef, and certain minerals would be suitable low-iodine components. The percentage composition of the diet was as follows: ground yellow corn, 61.1; wheat germ,[3] 30.0; refined cottonseed oil, 2.0; dried lean beef, 2.5; minerals, 4.0; and methionine, 0.4. Vitamin additions in milligrams per 100 gm of diet were: riboflavin 1.0, thiamine hydrochloride 0.75, niacin 5.0, *i*-inositol 30.0, pyridoxine hydrochloride 1.0, choline chloride 200, biotin 0.01, folic acid 0.01, calcium pantothenate 5.0, *p*-aminobenzoic acid 30.0, 2-methyl-1,4-naphthoquinone 0.50, α-tocopherol 25, vitamin A acetate 0.05, and calciferol 0.005. The mineral mixture, prepared from compounds shown to be low in iodine, consisted of (in parts by weight) sodium chloride 4.43, magnesium sulfate (anhydrous) 6.78, disodium phosphate 8.86, monopotassium phosphate 19.03, potassium chloride 10.41, calcium phosphate monobasic 13.75, calcium lactate 33.07, ferrous ammonium sulfate 3.63, manganese sulfate 0.016, copper sulfate 0.025, and zinc chloride 0.024.

In preliminary studies young rats grew well on this diet. However, the lactation of mature rats was insufficient to support normal growth of the young. Replacement of 0.2% of corn with an "animal protein factor" preparation[4] re-

[2] Partially defatted, dehydrated, and pulverized; purchased from the Viobin Corporation, Monticello, Illinois.

[3] See footnote 2.

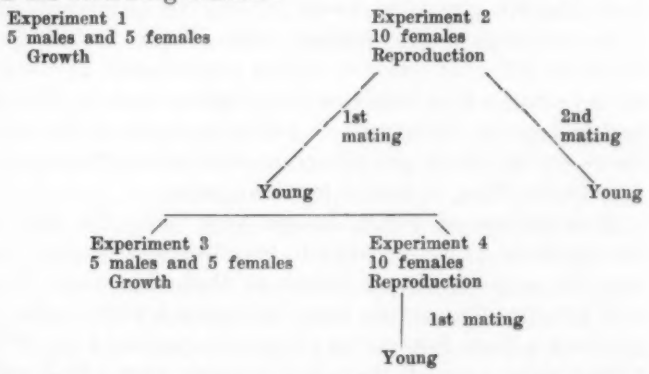
[4] Kindly supplied by Merck and Company, Incorporated.

sulted in a diet which supported adequate lactation. Rats in experiments 1 and 2 were grown on the diet without the "animal protein factor" preparation. However, the pregnant rats in experiment 2 and all rats in subsequent experiments were fed the diet containing this preparation.

Iodine determinations were made on the distilled water consumed by the rats (0.36 μg per liter), on each lot of the basal ration (average 25 μg per kilogram), and on some of the lots to which potassium iodide was added. The results of the latter were in good agreement with calculated values.

Design of experiments

The levels of iodine fed were 25, 50, 100, 225, and 525 μg per kilogram of ration. The different experiments are outlined in the following scheme:



In experiment 1, 40 weanling albino rats (38 to 46 gm) were divided equally with regard to litter, weight, and sex into 4 groups of 10 rats each and placed in individual wire cages. Rats in groups 1-1 to 1-4 (table 1) were fed diets containing 25, 100, 225, and 525 μg of iodine per kilogram of diet, respectively. Individual rats were weighed and food consumption data obtained at weekly intervals. After 6 weeks on the experimental diets, the rats were killed with chloroform and their thyroid glands removed. Sections were taken for

histological examination and the remaining tissue dried to constant weight in an air oven at 100°C. and analyzed for iodine by the Chaney ('40) method.

In experiment 2, 40 female weanling rats were divided into 4 groups and fed diets containing 25, 100, 225, and 525 μg of iodine per kilogram. After the animals had been on the experimental diets for 11 weeks, they were mated to male rats of approximately the same age which had been grown on the colony stock ration. Litters were reduced to 8 at birth and the thyroid glands removed from some of the animals sacrificed. Weanling rats from this experiment were used in experiments 3 and 4, and some were killed and their thyroid glands removed. As soon as the young rats were weaned, the adult females were again mated. After their second litters were weaned, the adult females were killed and their thyroid glands removed.

In experiment 3, weanling rats of the first litter from dams on different levels of iodine (experiment 2) were grown at the same iodine levels as their dams; namely, 25, 100, 225, and 525 μg per kilogram. In addition, some of the rats from dams on the 25 μg per kilogram level were grown on a diet containing 50 μg of iodine per kilogram.

Two groups of young female rats from the first mating (experiment 2) were grown to breeding age on diets containing the same levels of iodine as those fed their dams (25 and 525 μg). These rats were then mated with stock males to produce a third generation of rats (experiment 4). When the litters were weaned, the adult females were killed and their thyroid glands removed.

RESULTS

No significant differences in the rate of gain or food consumption were observed in growing rats on different levels of iodine (table 1). Enlarged thyroids, deep red in color, were observed in all of the rats fed the basal diet. The thyroids of rats in group 1-1 were significantly larger than those of rats in group 1-2 ($P<0.005$) and these, in turn, were sig-

nificantly larger than those of rats in group 1-3 ($P<0.005$). However, the thyroids of rats in group 1–3 did not differ significantly from those of rats in group 1–4 ($P>0.05$). The dry matter content of the thyroid varied directly with the iodine content of the diet. The thyroids from 9-week-old rats of the second generation on the basal diet (experiment 3) were larger and contained less iodine than those from rats of the first generation (table 1). However the level of iodine which prevented significant thyroid enlargement in 9-week-old rats of the second generation was the same as that for rats of the first generation. Using as a criterion the smallest amount of iodine necessary to prevent significant thyroid enlargement, the minimum iodine requirement of rats during growth was found to lie between 100 and 225 μg of iodine per kilogram of ration. The average daily iodine intake of rats on diets containing 100 and 225 μg of iodine per kilogram was 1.07 and 2.27 in experiment 1, and 1.36 and 2.99 in experiment 3.

Thyroids from growing rats (experiments 1 and 3) on the basal diet contained only a trace of colloid, the thyroid epithelium was of the tall columnar type and nearly all of the follicles showed evidences of hypertrophy and hyperplasia. Rats on the 50 μg per kilogram level of iodine had thyroids which contained slightly more colloid than those of rats on the lowest level of iodine; however, hypertrophy and hyperplasia were still apparent in some of the thyroids of this group. Thyroids from growing rats on the 100 μg per kilogram level of iodine contained moderate amounts of colloid and were not hyperplastic, but hypertrophy was noticeable. The histological appearance of thyroids from growing rats on the 225 μg per kilogram level of iodine was almost identical with that of thyroids from animals on the 525 μg per kilogram level. A small amount of hypertrophy was observed in these thyroids, but there was no noticeable increase in cell numbers and abundant colloid was present. More hyperplasia was observed in the thyroids of rats at the high levels of iodine in experiment 3 than in experiment 2.

TABLE 1

Effect of the iodine content of the diet on the weight and iodine and dry matter content of the thyroid glands of growing rats

EXPT. AND GROUP	GENERATION	IODINE CONTENT OF DIET	AVERAGE WEEKLY GAIN	AVERAGE WEEKLY FOOD CONSUMPTION	AVERAGE DAILY IODINE INTAKE	THYROID WEIGHTS PER 100 GM BODY WEIGHT		DRY MATTER CONTENT OF THYROID	IODINE CONTENT OF THYROID	
						Fresh	Dry		Average	Per cent of dry weight
		μg/kg	*gm*	*gm*	*μg*	*mg*	*mg*	%	*μg*	%
1 – 1	1st	25	25.8	76.3	0.27	11.6	2.73	26.1	0.61	0.011
1 – 2	1st	100	24.4	75.2	1.07	6.5	1.80	29.5	4.87	0.138
1 – 3	1st	225	23.4	70.5	2.27	5.5	1.64	30.4	6.55	0.220
1 – 4	1st	525	24.0	74.7	5.60	5.5	1.82	36.7	11.20	0.340
3 – 1	2nd	25	32.6	97.6	0.35	19.4	3.83	19.8	0.32	0.0035
3 – 2	2nd	50	29.4	89.6	0.64	10.8	2.29	21.2	1.19	0.0238
3 – 3	2nd	100	30.4	94.9	1.36	8.9	2.08	23.4	5.19	0.110
3 – 4	2nd	225	30.6	93.2	2.99	6.7	1.65	24.4	7.39	0.199
3 – 5	2nd	525	30.6	92.3	6.92	6.3	1.58	25.3	14.26	0.340

TABLE 2

Effect of different iodine levels on reproduction

EXPT. AND GROUP	IODINE CONTENT OF RATION	GENERATION OF ADULT FEMALES	LITTER	NUMBER OF PREGNANCIES	AVERAGE NUMBER OF LIVE YOUNG PER LITTER	AVERAGE LITTER WEIGHT AT BIRTH	AVERAGE NUMBER OF RATS WEANED PER LITTER	AVERAGE RAT WEIGHT 21 DAYS AFTER BIRTH		AVERAGE GAIN OF ADULT FEMALES DURING LACTATION
								All litters	Litters containing 8 rats	
	μg/kg					*gm*		*gm*	*gm*	*gm*
2 – 1A	25	1st	1st	9	11.3	66.1	7.8	38.3	39.2	27
2 – 2A	100	1st	1st	10	9.3	51.8	6.4 [1]	37.8	37.8	22
2 – 3A	225	1st	1st	10	8.8	48.7	7.7	38.6	38.1	22
2 – 4A	525	1st	1st	9	8.4	42.9	6.4 [2]	41.4	40.9	18
2 – 1B	25	1st	2nd	8	12.1	68.6	6.6	42.4	40.9	12
2 – 2B	100	1st	2nd	10	10.2	57.0	7.1	40.7	39.7	9
2 – 3B	225	1st	2nd	9	10.2	57.4	7.4	44.2	43.1	16
2 – 4B	525	1st	2nd	9	8.8	52.4	7.1	42.9	42.8	6
4 – 1	25	2nd	1st	10	9.4	53.0	6.8	42.2	41.4	11
4 – 2	525	2nd	1st	10	9.1	45.6	6.2 [3]	42.1	39.4	9

[1] All rats in two litters died.

[2] All rats in one litter died.

In the reproduction experiments, healthy young animals were produced in normal numbers on the basal diet containing 25 μg per kilogram of iodine. The number of live young per litter was actually greater at the lowest level of iodine than at higher levels. Although the average number of live young per litter in the first and second litters from dams on the basal diet in experiment 2 was not significantly greater than that from dams at the 100 μg per kilogram level ($P > 0.05$), litters at the basal level were significantly larger than litters at the 225 μg per kilogram level ($P < 0.05$) and the 525 μg per kilogram level ($P < 0.01$).

The differences in thyroid weights of rats at different levels of iodine in the reproduction studies (experiments 2 and 4) were similar to those observed in the growth studies. Thyroids from newborn, weanling, and adult female rats at the basal level of iodine were much larger than those from rats at higher levels of iodine. Some thyroid enlargement was observed in all groups of rats at the 100 μg per kilogram level, but thyroids from rats at the 225 μg per kilogram level were not significantly larger than those from animals at the 525 μg level.

Weanling rats of the second litter from dams at the basal level of iodine had slightly larger thyroids than weanling rats of the first litter (table 3); however, weanling rats from dams of the second generation on the basal diet (experiment 4) did not have larger thyroids than weanling rats from dams of the first generation (experiment 2).

The thyroid weight per 100 gm body weight varied indirectly with the age of the rats. The fresh thyroid weights per 100 gm body weight of newborn (less than one day old), weanling (21 days), growing (9 weeks) and adult female (autopsied at the time the young were weaned) rats at the highest level of iodine were approximately 15, 10, 6, and 5.5 mg, respectively.

The iodine concentration in the thyroids of rats at the basal level of iodine was extremely small. Weanling rats at the 25 and 100 μg per kilogram levels had higher percentages

of iodine in dry thyroids than their dams; however, at the 525 μg level the per cent iodine in dry thyroids from weanling rats was less than that of their dams (tables 3 and 4).

Thyroids from newborn rats at the basal iodine level had the general appearance of embryonic thyroid glands. Instead of the typical follicular arrangement of the more mature thyroid, the epithelium had the appearance of masses of cells in disorderly arrangement, showing considerable activity and hyperplasia. The histological appearance of thyroids from newborn rats at higher levels of iodine was more typical of the mature gland. However, the colloid in the thyroids of newborn rats at the 100 and 225 μg per kilogram levels was still extremely thin and scant, and only a moderate amount of colloid was present in the gland at the 525 μg level. Both hypertrophy and hyperplasia were still observable in the thyroids of newborn rats at the 100 μg per kilogram level; however, only slight hypertrophy was noticed at the 225 μg level and neither hypertrophy nor hyperplasia was noticeable at the 525 μg level.

TABLE 3

Weight and iodine content of thyroid glands of young rats

EXPT. AND GROUP	IODINE CONTENT OF RATION	GENERATION ON RATIONS	LITTER	FRESH THYROID WEIGHT PER 100 GM BODY WEIGHT OF RATS AT BIRTH	WEANLING RATS (21 DAYS OF AGE)				
					Thyroid weight per 100 gm body weight		Dry matter content of thyroid	Iodine content of thyroid	
					Fresh	Dry		Average	Per cent of dry weight
	μg/kg			mg	mg	mg	%	μg	%
2 – 1A	25	2nd	1st	40.5	19.3	3.82	20.0	0.185	0.0118
2 – 2A	100	2nd	1st	25.7	11.8	2.85	24.2	1.19	0.106
2 – 3A	225	2nd	1st	18.4	9.8	2.64	27.2	1.53	0.142
2 – 4A	525	2nd	1st	15.5	10.4	2.88	27.6	2.45	0.198
2 – 1B	25	2nd	2nd	40.7	24.2	4.77	19.9	0.104	0.00506
2 – 2B	100	2nd	2nd	...	12.1	3.37	28.0	1.23	0.0885
2 – 3B	225	2nd	2nd	...	11.6	3.35	29.0	2.37	0.158
2 – 4B	525	2nd	2nd	...	10.7	3.08	28.0	2.05	0.156
4 – 1	25	3rd	1st	33.0	18.2	3.85	21.3	0.147	0.0093
4 – 2	525	3rd	1st	14.3	10.2	2.97	30.6	1.44	0.107

The lactating condition of adult females at slaughter may have had some effect on thyroid appearance, since it is well known that certain relationships exist between the thyroid gland and milk production. Both hypertrophy and varying degrees of hyperplasia were found in their thyroids at all levels of iodine intake. With the exception of differences in gross size, the thyroids of rats at the 100 μg per kilogram level resembled those of animals at the basal level. Only a trace of colloid was present in these thyroids, the epithelium was of the tall columnar type, and both hypertrophy and hyperplasia were evident. Those at the 225 and 525 μg levels contained moderate to large amounts of colloid, with a tendency for more at the higher level. Epithelial hypertrophy and slight hyperplasia were evident in at least some areas of nearly all of the glands.

TABLE 4

Weight and iodine content of thyroid glands of adult female rats

EXPT. AND GROUP	IODINE CONTENT OF DIET	GENERATION ON DIETS	THYROID WEIGHT PER 100 GM BODY WEIGHT		DRY MATTER CONTENT OF THYROID	IODINE CONTENT OF THYROID	
			Fresh	Dry		Average	Per cent of dry weight
	μg/kg		*mg*	*mg*	%	*μg*	%
2 – 1	25	1st	17.16	3.28	19.3	0.320	0.0034
2 – 2	100	1st	7.04	1.54	21.9	1.09	0.0244
2 – 3	225	1st	5.91	1.30	22.0	6.10	0.165
2 – 4	525	1st	5.48	1.43	26.0	11.11	0.271
4 – 1	25	2nd	18.07	3.44	19.1	0.455	0.0044
4 – 2	525	2nd	5.83	1.36	23.4	11.41	0.306

DISCUSSION

While the iodine content of the basal diet (25 μg per kilogram) was not so low as that used by Levine et al. ('33a), it was sufficiently complete in other nutritional components to produce good growth, reproduction, and lactation. There was no evidence of a deficiency of any nutrient other than

iodine in three successive generations. Accordingly, the picture presented is thought to represent uncomplicated iodine deficiency.

The amount of iodine needed to prevent significant thyroid enlargement in rats grown on the diets used in these experiments is, nevertheless, in good agreement with that found by Levine et al. The iodine content of the diets (110 and 207 μg per kilogram), which corresponded to the minimum daily iodine intake of approximately 1 to 2 μg needed to prevent any significant thyroid enlargement according to studies by Levine et al., was within the range found in the present investigation. However, the minimum daily iodine intake needed to prevent thyroid enlargement in these studies was higher than that reported by Levine et al., possibly because of the higher growth rate and food consumption. Rats which were fed diets containing the "animal protein factor" in experiment 3 grew faster and therefore consumed more food than the rats in experiment 1, and these in turn grew faster and consumed more food than the rats in the studies by Levine et al.; however, the minimum iodine level needed in the diets to prevent thyroid enlargement was approximately the same in all of these experiments.

The iodine requirement during pregnancy and lactation was essentially the same as that during growth, when expressed as percentage in the diet necessary to prevent thyroid enlargement. The embryonic appearance of the thyroids of newborn rats from dams on the low level of iodine indicated that a deficiency of iodine in the diet of the dams interfered with the normal differentiation at the fetal thyroid. The low iodine content and histology of the thyroids from adult females on the two lowest levels of iodine indicate that lactation tended to deplete iodine reserves. However, even under the heavy demands of pregnancy and lactation, lowering the iodine level of the diet to 25 μg per kilogram did not noticeably interfere with the metabolic functions of the rat. Rats on the basal diet

actually produced more live young per litter than animals on higher levels of iodine.

SUMMARY

With rats grown for three successive generations on diets containing 25 to 525 μg of iodine per kilogram, the minimum dietary level needed to prevent thyroid enlargement was found to lie between 100 and 225 μg per kilogram. The growth rates of rats fed the different levels of iodine did not differ significantly.

Lowering the iodine content of the diet of female rats to 25 μg per kilogram did not interfere with reproduction or lactation; however, the thyroids of the newborn from dams at this level had the appearance of embryonic glands.

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PROPYLTHIOURACIL, THYROID, AND DIETARY LIVER INJURY [1]

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SIXTEEN FIGURES

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György and Goldblatt ('45) have reported that thiouracil and, to a lesser extent, thiourea, exerted a beneficial effect in the prevention of dietary cirrhosis in rats. It was suggested that the mechanism involved was related to the action of these drugs on thyroid activity. In a later report ('48) the same authors presented "indirect circumstantial" evidence that various goitrogenic compounds, including propylthiouracil, exerted a beneficial effect in preventing cirrhosis, and observed that while rats fed propylthiouracil ate on the average only 52% of the amount of food taken by control animals, their gain in body weight was greater and their survival rate higher.

When normal rats are fed a diet containing propylthiouracil, a considerable decrease in intake of food is always observed during the first few weeks. The relationship of the amount of food ingested to the production of dietary fatty livers, "hemorrhagic" kidneys and even to survival rate, in experiments involving lipotropic factors, made it seem advisable to control this factor by pair feeding. Accordingly experiments of varied duration employing both "pair feeding" and "ad libitum" feeding were performed, in order to study the

[1] These experiments were supported in part by grants from the Ontario Cancer Treatment and Research Foundation and the National Cancer Institute of Canada.

effect of propylthiouracil on the deposition of fat in the liver and on the production of cirrhosis.

EXPERIMENTAL

Short-term experiments

Twenty female rats of the Wistar strain bred locally and weighing between 50 and 60 gm were divided into two groups of 10. They were placed in individual cages and allowed water ad libitum. One group was fed a diet which permitted good growth but was deficient in choline and its precursors. The

TABLE 1

Effect of propylthiouracil (PTU) on acute choline deficiency in female rats

GROUP	NO. OF RATS	PTU % IN DIET	DURATION OF FEEDING	AVE. BODY WT.		AVE. FOOD INTAKE	AVE. THYROID WT.	AVE. LIVER WT.	AVE. TOTAL LIPIDS
				Initial	Final				
			days	*gm*	*gm*	*gm/day*	*mg*	*gm*	*% wet liver wt. ± S.D.*
A	10	0.02	9	48	54	3.6[2]	20.6	4.3	28.1 ± 3.9
	10	0	9	48	57	3.8[2]	6.2	4.6	25.9 ± 5.0
B	9[1]	0.02	9	72	93	7.0[2]	43.6	7.0	27.2 ± 5.6
	7	0	9	69	93	7.0[3]	9.8	6.7	21.9 ± 3.8
C	10[1]	0.02	14	145	153	9.2[3]	..	7.0	10.4 } group
	5	0	14	140	166	11.3[3]	..	9.9	24.5 } group

[1] PTU was started one week before the commencement of choline-deficient diet.
[2] Pair-fed.
[3] Ad libitum.

other was given the same diet with 0.02% propylthiouracil[2] added. The first group was "pair-fed" with respect to the second; that is, each rat was allowed only the average amount consumed the day previously by the rats of the group receiving propylthiouracil. After 9 days the animals were sacrificed and chemical and histological examinations of the livers were carried out (table 1, group A).

The experiment was repeated using female rats of a similar weight range, but this time the feeding of propylthiouracil

[3] The British Drug Houses (Canada) Limited.

was started one week prior to the commencement of the hypolipotropic diet. The purpose of this was to ensure that the drug had produced an effect before the diet low in choline was given. Nine days after starting the hypolipotropic diet the animals were sacrificed and the livers were removed for chemical and histological examination (table 1, group B).

Older female rats of greater weight (137 to 154 gm) were given the hypolipotropic diet (ad libitum) with and without propylthiouracil, and after 14 days were sacrificed for histological examination. Ten animals were given the hypolipotropic diet with propylthiouracil and 5 served as controls. The giving of propylthiouracil was started a week prior to the commencement of the hypolipotropic diets, as in the previous experiment (table 1, group C).

Long-term experiments

In the first series of experiments female rats of 124 to 150 gm were fed the basal hypolipotropic diet with or without supplements according to the following schedule:

Group 1 — Basal diet, pair-fed with group 2, 10 rats.

Group 2 — Basal diet + 0.02% propylthiouracil, 10 rats.

Group 3 — Basal diet + 0.1% thyroid,[3] pair-fed with group 2, 10 rats.

Group 4 — Basal diet fed ad libitum, 10 thyroidectomized rats.

Group 5 — Basal diet ad libitum, 10 rats.

Group 6 — Basal diet + 0.1% thyroid fed ad libitum, 15 rats.

Groups 1 and 2 were sacrificed after 6 months, the others after three months. Several deaths had occurred in groups 3 and 4 and the animals were in poor condition.

All rats were kept in individual metal cages so that intake of food could be followed daily. Changes in body weight were recorded weekly.

In the second series of experiments male and female rats (60 to 110 gm) were kept in group cages and fed ad libitum.

[3] Dried thyroid (0.3% iodine), Parke, Davis and Co., Ltd., Walkerville, Ontario.

Fifty-four females and 54 males were given 0.02% propylthiouracil incorporated in the hypolipotropic diet; 20 males and 20 females received the diet without the supplement. The animals were sacrificed at intervals, the final group of 19 animals treated with propylthiouracil being killed after 15 months of treatment.

The diet

The basal diet used was low in choline and methionine, but when choline was added it produced a good rate of growth. The basis of the diet was peanut meal,[4] supplemented with a small amount of "vitamin-free" casein. The diet composition was as follows: Peanut meal 30%, casein 6%, salts 4% (Beveridge and Lucas, '45), sucrose 39%, "vitamin powder" 1%, beef fat 15%, corn oil 5%, alpha-tocopherol acetate 0.010%, cod liver oil concentrate 0.015%. The "vitamin powder" was of such a composition that the intake per 10 gm of diet was as follows: Biotin 3 μg, thiamine hydrochloride 50 μg, riboflavin 25 μg, pyridoxine hydrochloride 20 μg, calcium pantothenate 100 μg, nicotinic acid 100 μg, folic acid 5 μg, 2-methyl 1-4 naphthoquinone 10 μg, inositol 5 mg, para-aminobenzoic acid 1 mg. The cod liver oil concentrate supplied at least 300 I.U. vitamin A and at least 75 I.U. vitamin D per 10 gm of diet.

RESULTS

Short-term series

Extensive deposition of fat occurred in all weanling rats receiving the hypolipotropic diet, whether propylthiouracil was present in the diet or not (table 1, groups A and B). The extent of deposition and distribution of fat (figs. 1, 2) was fairly similar in the two groups.

[4] Before processing, batches of the peanut meal contained from 1.8 to 2.9 mg/gm total choline. The meal was extracted successively in 50, 70 and finally 95% alcohol, and between extractions was filtered as dry as possible in a filter press. After this procedure estimations for total choline indicated a content of from 0.2 to 0.3 mg/gm.

In older rats (fed ad libitum) the amount of fat in the liver was less in the group receiving propylthiouracil (table 1, group C). In this group the daily food intake was 9.2 gm compared to 11.3 gm in the control animals. A difference in distribution of fat in the lobules was observed. In the sections from the group receiving propylthiouracil, fat was in fine globules and appeared to be almost entirely intracellular (fig. 3). In the case of the control animals (fig. 4), in addition to fine globules of fat within the cells, fat had been deposited in larger accumulations, some in "extracellular cysts."[5]

Long-term series

When the dietary intake of a group (table 2, group 1) receiving the basal diet was restricted by pair-feeding with a group (table 2, group 2) receiving propylthiouracil, the fat content of the livers was still greatly elevated. No increased fibrosis or reticulin could be seen in the livers of either group (table 4). The distribution of fat in the liver differed, in that fatty cysts were a prominent feature in the animals fed the basal diet, i.e., the fat was in large part extracellular, while in the animals fed the diet containing propylthiouracil the fat was predominantly intracellular, and fatty cysts appeared less frequently (figs. 5, 6).

This difference was especially noticeable in the livers of thyroidectomized animals (table 2, group 4), where fatty cysts were very uncommon although the fat content as estimated chemically was high (ave. 25.7%, fig. 7). The food intake of thyroidectomized rats was 6 gm per day and the average increase in body weight was low. In group 3 (basal diet with 0.1% thyroid, pair-fed with the group receiving propylthiouracil) the fat content as estimated chemically was extremely variable, averaging 15.6 $\pm$ s.d. 10.4% of the wet liver weight. Histologically fat appeared to be chiefly in

[5] In the remainder of this paper "fatty cyst" or "extracellular" cyst is used without further description in the same sense as Hartroft ('50) originally used "lipodiastaema." In early stages it is difficult or impossible to state definitely that such fat is extracellular. Later, it seems clear that it is truly extracellular.

the central part of the lobule largely in extracellular fatty cysts (fig. 8).

When a similar diet (i.e., with thyroid, group 6) was fed ad libitum for the same period (three months) the distribution of fat was similar, although the fatty cysts were more frequent and were often larger (fig. 10). In this case, in the central parts of the lobules in proximity to cystic spaces, thin strands of reticulin could be seen encircling nuclei (or cells with very little cytoplasm), and cysts apparently empty

TABLE 2

Effects of propylthiouracil,[1] thyroid,[2] and thyroidectomy on chronic choline deficiency in female rats

GROUP	NO. OF RATS	TREATMENT	DURATION OF FEEDING	AVE. BODY WT.		AVE. FOOD INTAKE	AVE. THYROID WT.	AVE. LIVER WT.	AVE. TOTAL LIPIDS
				Initial	Final				
			months	*gm*	*gm*	*gm/day*	*mg*	*gm*	*% wet liver wt. ± S.D.*
1	9	....	6	135	203	6.0	14.5	11.0	29.2 ± 8.0
2	9	Propyl-thiou-racil	6	130	165	6.0	89.0	7.7	21.4 ± 10.5
3	9	Thyroid	3	120	126	6.0	...	7.1	15.6 ± 10.4
4	6	Thyroid-ectomy	3	138	169	6.0	...	8.6	25.7 ± 13.6
5	10	....	3	122	185	10.7	24.5	15.4	34.9 } group
6	12	Thyroid	3	124	170	12.6	17.1	20.1	29.4 }

[1] Propylthiouracil 0.02% in the basal diet.
[2] Dried thyroid 0.1% in the basal diet.

or partially emptied of fat (figs. 11, 12, 13). This appearance differed from that of the livers of rats fed the basal diet ad libitum (group 5) in that the cysts were often larger and were frequently present only in the central part of the lobules. Although considerable variation occurred, stainable fat was present in only minimum amounts, even *within* hepatic cells in the peripheral part of the lobules. In the controls fatty cysts and intracellular fat often extended to the outermost parts of the lobule (fig. 9). In the two groups the *apparent* increase in reticulin was similar. From the appearance of

the sections the difference in number of cysts would seem to account for the difference in fat content between control and thyroid-treated animals (table 2). The average daily food consumption of the animals receiving thyroid was higher by 2 gm than that of the controls.

In the groups of animals kept in group cages and fed the diets with and without propylthiouracil ad libitum for prolonged periods, a considerable variation in gain in body weight and in amount of fat deposited in the liver was observed. During the first 4 or 5 months the animals receiving propylthiouracil, although eating less, gained almost as much

TABLE 3

Effect of propylthiouracil (PTU) on fat deposition in chronic choline deficiency
(Ad libitum feeding)

NO. OF RATS	DURATION OF FEEDING	PTU, % IN DIET	AVE. BODY WT.		AVE. THYROID WT.	AVE. LIVER WT.	AVE. TOTAL LIPIDS
			Initial	Final			
	months		*gm*	*gm*	*mg*	*gm/100 gm body wt.*	*% wet liver wt. ± S.D.*
6	6	0.02	79	128	63.5	4.1	12.8 ± 11.5
6	6	0	64	217	11.7	9.3	35.8 ± 11.1
10	12	0.02	69	140	140.5	6.6	34.8 [1]
8	12	0	70	240	...	8.2	24.2 [1]
7	15	0.02	110	189	111.2	5.2	23.3 ± 2.4

[1] Livers were pooled for fat estimation.

weight as and appeared to be in better condition than the controls. Ultimately (table 3) the body weights of survivors in the control group were higher than those of animals in the propylthiouracil group. After 6 months the amount of fat, as estimated chemically in a group of animals picked at random and receiving propylthiouracil, was much less than in the controls. At 12 months these relative positions were less clear, the livers of both groups being very fatty. In the early stages of the experiment the distribution of fat agreed in general with what has been stated above; that is, in the animals receiving propylthiouracil, fat tended to be intracellular, while in the controls fatty cysts were a more promi-

nent feature. At 12 and 15 months many large fatty cysts were present in the livers of both groups.

During the first 6 months it was obvious that bands of "fibrous tissue" were more prominent in the livers of animals receiving the basal diet than in livers of rats receiving propylthiouracil (table 4). However, by 12 months moderately severe fibrosis had developed in each group (figs. 14, 15, 16). At 15 months most of the animals receiving propyl-

TABLE 4

Effect of propylthiouracil (PTU) on liver cirrhosis

TREATMENT	NO. OF RATS	METHOD OF FEEDING	DURATION IN MOS.	HISTOLOGICAL FINDINGS		
				Fatty infiltration	Fibrosis	Ceroid
Propyl-thiouracil	9	Pair	6	Severe	..	..
Controls	9	Pair	6	Severe	..	..
Propyl-thiouracil	11	Ad lib.	6	Slight	±	..
Controls	8	Ad lib.	6	Severe	++	..
Propyl-thiouracil	20	Ad lib.	12	Moderate to severe	++	±
Controls	20	Ad lib.	12	Moderate to severe	+++	++
Propyl-thiouracil	19	Ad lib.	15	Slight to severe	+ to +++	++

thiouracil had extensive fibrosis in addition to considerable stainable fat in extracellular cysts. Five of the rats in this group differed from the others in becoming very obese. At autopsy, the tremendous deposits of subcutaneous and depot fat in these rats were impressive. The livers of these obese rats differed from those of the remaining 14 animals in containing very little fat and in showing no increase of fibrous tissue. The whole picture suggested that some major change in endocrine balance had occurred in the obese rats. Its significance is being studied.

The kidney lesions (widespread cortical tubular degeneration with hemorrhage) produced by feeding the diet low in choline appeared to be ameliorated by propylthiouracil, and accentuated by feeding the thyroid supplement. This was apparent in all the experiments of short duration. The differences appeared to be of degree rather than type. In groups fed ad libitum, in which the intake of food was greatest, kidney lesions were most severe.

DISCUSSION

Propylthiouracil exerts a pronounced effect on food consumption and on rate of growth. It has been observed previously that when fed ad libitum animals receiving propylthiouracil consume less food than, but show an increase in body weight comparable to that of, controls fed a similar diet without the drug. After a period of weeks the rate of growth is adversely affected by propylthiouracil, although in pair-feeding experiments it can be demonstrated that this effect is largely but not entirely because of the lower caloric intake. The effects of prolonged feeding of propylthiouracil are not well known. It is not easy to foresee the end results of decreased thyroid activity, decreased caloric intake and other less well recognized actions of the drug. Likewise a number of important but poorly understood factors (environmental temperature, growth rate, dietary interrelationships, adrenal function, and so forth) affect the lesions observed after feeding diets low in lipotropic factors. The experiments reported here clarify some of the effects of propylthiouracil on the deposition of fat in the liver and the "prevention" of dietary cirrhosis, but also illustrate the complexity of these relationships. (See also Handler and Follis, '48.)

In the present experiments, if the consumption of food with and without propylthiouracil is compared, it is found that in weanling rats the intakes did not differ greatly. Therefore, one could anticipate that the content of liver fat might be similar, and this result was observed. When severely hypolipotropic diets are fed ad libitum to young rats, such an

experiment is self-limited, for after 7 to 10 days many of the animals die of uremia.

Consumption of food containing propylthiouracil usually falls off over a period of several weeks. Thus it might be expected that any effect of food consumption on the level of liver lipids would appear only after a period of weeks. When two groups of older rats (one of which had been given propylthiouracil for a week preceding the experiment) were fed the same hypolipotropic diets (i.e., with and without propylthiouracil) ad libitum for two weeks, the fat content of the livers of rats receiving propylthiouracil averaged 10.4% compared to 24.5% in the control group. The food intake averaged 2 gm per day higher in the latter group.

In the experiment lasting 6 months in which the food intake of treated and control animals was made equal, the group fed propylthiouracil had a lower content of liver lipids ($21.4 \pm 10.5\%$ compared to $29.2 \pm 8\%$), but this difference was not statistically significant ($P = 0.1 - 0.2$).

These results suggest that a principal effect of propylthiouracil on deposition of fat in the liver is indirect, viz., that the drug acts by causing a decrease in the caloric intake. The action of propylthiouracil is not explained so simply, however, because metabolic rate and rate of skeletal growth as well as intake of food are all decreased when it is ingested. It could be expected that alterations of these factors might influence the amount of fat in the liver at any given time.

When the metabolic rate is increased by giving thyroid substance to rats receiving a hypolipotropic diet, the variation between rats in content of liver lipids increases. The average value is reduced, although with the particular diet employed a high dosage of thyroid is necessary before the content of lipids in the liver approaches normal. Such an amount of thyroid affects the rat adversely, preventing growth and increasing the mortality rate. The livers of some rats in a group so treated may still have an increased fat content. Histologically, much of the fat is seen to be present

in fatty cysts (extracellular) disposed near the central part of the lobule. With lower doses of thyroid (0.1%) this distribution is still apparent although the level of lipids may be elevated greatly and stainable fat may be seen throughout the lobule.

By exposing rats on the same hypolipotropic diet to a cold environment (which results in a metabolic rate higher than that of rats fed 0.8% thyroid) but in which increased thyroid activity apparently plays only a small part (Sellers and You, '50), the level of liver lipids may remain within or close to normal limits (Sellers and You, '49). Any stainable fat present appears as an increase in intracellular fat globules. The mechanism involved in the two cases would appear to differ, and it is suggested that the difference lies in the action of the thyroid hormone on deposition of liver lipids.

It has been stated above that the distribution of fat within the liver lobules differs, depending in part on the activity of the thyroid. Thus, in thyroidectomized animals or in rats given propylthiouracil, there is a fine globular intracellular distribution in all parts of the lobule, and although the fat content by chemical examination may be as high as 20 to 25%, few extracellular fatty cysts are seen. This type of distribution also occurs to some extent when intake of the hypolipotropic diet is restricted in amount, but in such a case (unless the restriction is very severe) extracellular fatty cysts still remain a prominent histological feature.

When small doses of thyroid were fed for several months, "cysts" containing fat could be seen near the central vein although the cells in the periphery were sometimes devoid of stainable fat.

It is difficult to explain why fatty cysts may occur centrally when the peripheral part of the lobule is relatively free of stainable fat. Hartroft ('50) has observed and reported that fatty cysts are formed by the coalescence of intracellular fat globules with one another and finally by the rupure of the cytoplasmic walls of two adjacent fat-laden cells. The subsequent rupture of other cells or cysts into those pre-

viously formed may increase the volume of the cyst to a large degree. The first site in choline deficiency in which stainable fat is seen, and the last site from which it disappears on restoration of choline to the diet, is near the central vein. It seems reasonable to think that as the amount of fat increases in this region fatty cysts might also be formed here most easily. After thyroidectomy, however, the fat content may be very high but all visible fat is distributed in fine globules within cells in all parts of the lobule.

It has been suggested by Hartroft that the emptying of fat from, and consequent collapse of, many of these "cystic" spaces gives rise to the components of the cellular "fibrous" tissue seen in dietary cirrhosis. It seems likely that in this type of cirrhosis the "fibrous" tissue described by numerous workers is indeed formed in this way. It is suggested that the numerous undifferentiated cells, which have often been described, trace their origin to the degenerated liver cells which once made up the walls of the cystic spaces. The persistence of cellularity and the small amount of collagen seen in the fibrous tissue of dietary cirrhosis would seem to support this view.

In the earlier stages, which have been studied most, we believe that there is little *true* fibrosis, that the cellular "fibrous bands" are composed in large part of atrophied and compressed liver cells, enveloped by reticulin and packed closely together. This would result in a local increase of reticulin fibres but until regeneration of lobules took place there would be no increase of reticulin in the entire liver.

It has been observed frequently that human cirrhosis may be associated with long standing hyperthyroidism. Our experiments demonstrate that in a type of experimental cirrhosis associated with excessive deposition of fat in the liver, the activity of the thyroid influences in some way the manner of deposition of fat. While by chemical analysis the fat content may be relatively low, it is distributed in such a way within the lobule that the sequence of events leading to fibrosis (as described by Hartroft) is favoured. This

observation may throw some light on the pathogenesis of similar conditions encountered in the clinic.

What is the fundamental cause of this effect of thyroid? The high concentration of lipids which may be present in the livers of thyroidectomized animals *without* formation of extracellular fatty cysts demonstrates that the level of liver lipids is not in itself the critical factor. No information is available as to the effect of type of lipid (for instance, glyceride, cholesterol ester, phospholipid) or of rate of "turnover" of lipids on the formation of fatty cysts. It would appear that rate of metabolism in relation to the caloric requirement is important, but the relationship is not clear since all the relevant essential data are not yet available.

Dietary cirrhosis is not prevented by feeding propylthiouracil but its time of appearance is delayed. The effect of propylthiouracil in "preventing" dietary cirrhosis is accounted for partly by the reduced caloric intake. Restricting the food intake of control animals to the same degree also delayed the appearance of cirrhosis. The presence of thyroid hormone influences the type of deposition of fat in the liver, and thus propylthiouracil, by preventing the synthesis of thyroid hormone, also exerts an effect more direct than that due to changed caloric intake on the production of this type of cirrhosis.

SUMMARY

The effect of propylthiouracil in retarding the appearance of dietary (low choline) cirrhosis is partly due to the reduced caloric intake of rats given the drug. Restricting the food intake of control rats to the same degree also delayed the appearance of cirrhosis.

The thyroid hormone influences the site and manner of deposition of fat in the liver lobule. Fat tends to be laid down throughout the lobule as *intracellular* lipid in the livers of rats with deficient thyroid function. With normal thyroid function, or especially if thyroid is fed to rats, lipid tends to accumulate in the central part of the lobule, and even when

the level of fat in the liver is low, extracellular fatty cysts are frequently formed.

The etiological relationship of extracellular deposition of fat to the production of dietary cirrhosis is discussed. It is suggested that in addition to its effect on caloric intake, propylthiouracil also exerts a more direct effect in retarding the development of cirrhosis, by preventing the synthesis of thyroid hormone and thus influencing the manner of deposition of fat in the liver.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Jessie Ridout and Dr. Jean Patterson for carrying out the estimations of liver lipids. Part of the experimental work was done in a collaborative study with Professor J. K. W. Ferguson in the Department of Pharmacology. The association with and timely advice from Professor C. H. Best and Professor C. C. Lucas have stimulated and helped us greatly. The histological material was prepared under the direction of Professor W. Stanley Hartroft, to whom we extend our thanks.

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PLATES

PLATE 1

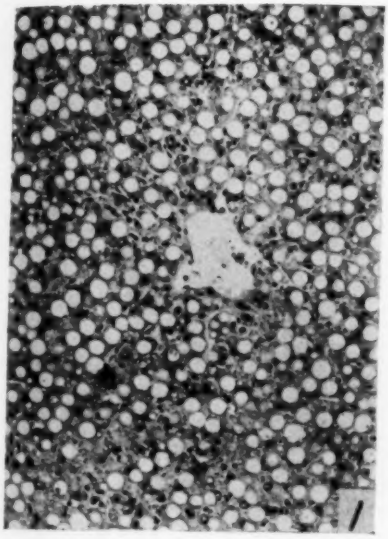
EXPLANATION OF FIGURES

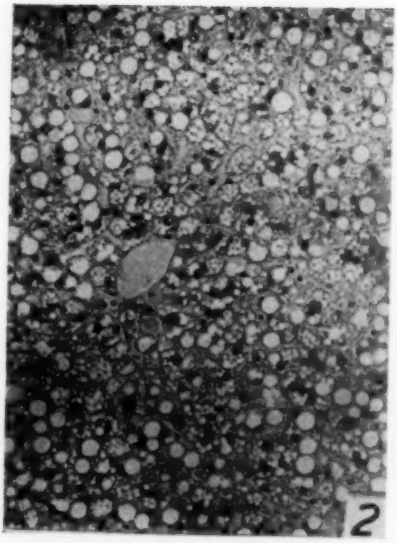
1 and 2 Figure 1 shows the great extent and general distribution of fat within the liver lobule. Weanling rats pair-fed the hypolipotropic diet for 9 days. With propylthiouracil added (fig. 2) the appearance is fairly similar, although the spaces which contained fat are frequently smaller and often obviously intracellular. (Hematoxylin and eosin, × 160.)

3 and 4 Figure 3 shows the liver of an older rat, fed the hypolipotropic diet with propylthiouracil for 14 days. Intracellular fat is distributed generally throughout the lobule.

Figure 4 In the control (without propylthiouracil, fed ad libitum) fatty cysts have developed near the central vein and extend into the mid zone. (Orange Red 0 × 160.)

PLATE 1





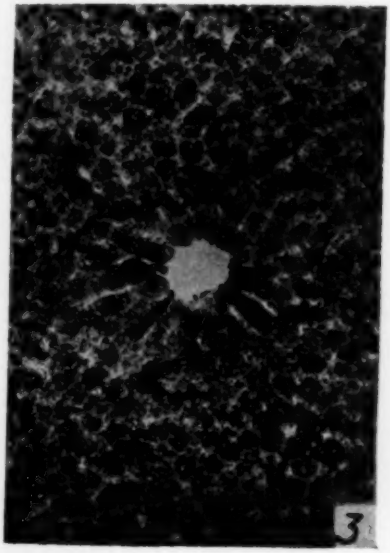
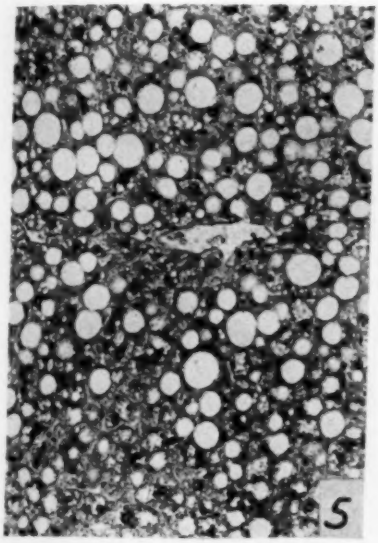


PLATE 2

EXPLANATION OF FIGURES

5, 6, 7 and 8 After 6 months' restricted feeding of the hypolipotropic diet (pair-fed with respect to propylthiouracil group) there are many large fatty cysts in the liver but no fibrosis is seen (fig. 5). With propylthiouracil, fat is predominantly intracellular but some large fatty cysts have developed. No fibrosis is seen. After thyroidectomy, few fatty cysts develop (none in section shown in fig. 7). With 0.1% dried thyroid added fatty cysts are distributed centrally (fig. 8). Sections of thyroidectomized and thyroid fed animals taken after three months. (Hematoxylin and eosin, × 160.)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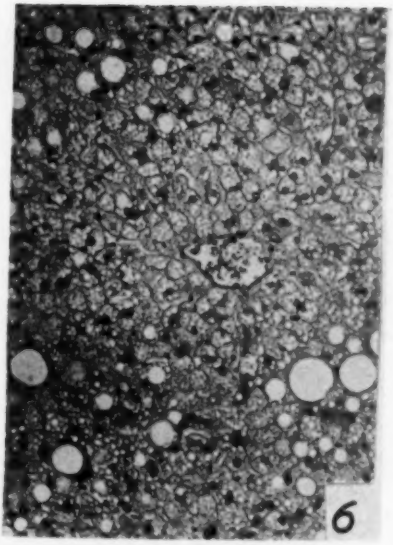
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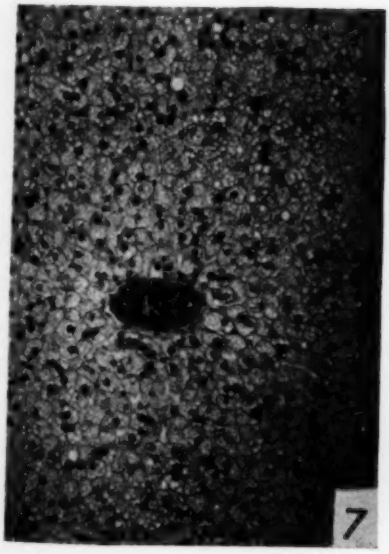
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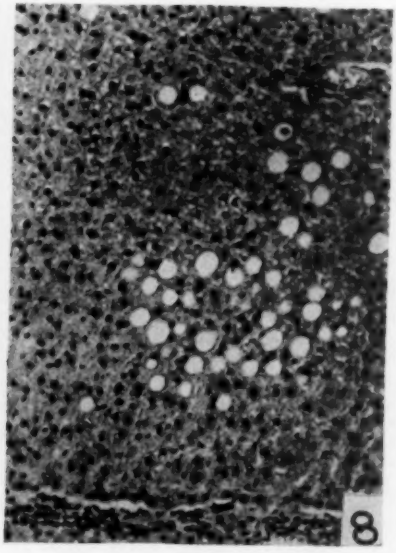
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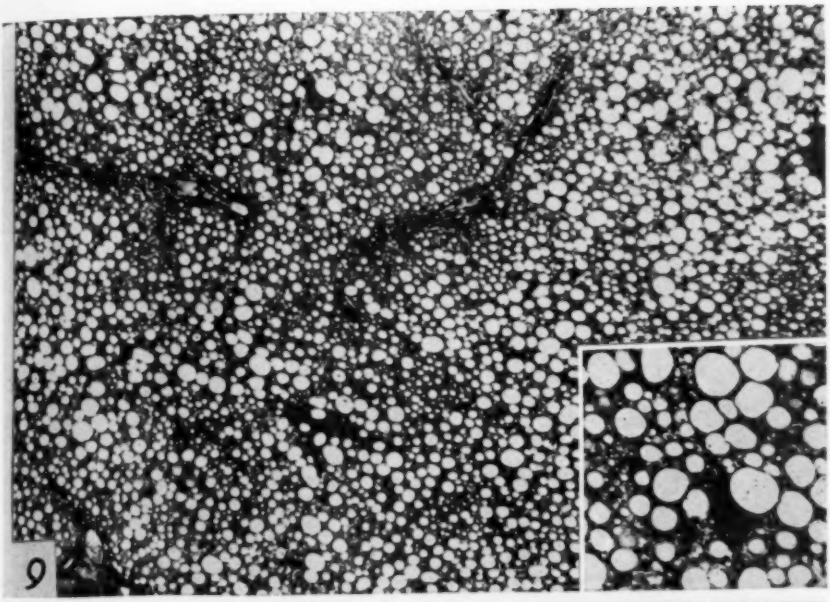
PLATE 3

EXPLANATION OF FIGURES

9 and 10 Figure 9 shows the general distribution of fatty cysts in the liver of a rat fed the hypolipotropic diet ad libitum for three months. Note that fatty cysts are distributed generally throughout the lobules.

Figure 10 is a section from the liver of a rat fed the hypolipotropic diet with 0.1% thyroid added. Note that the cysts are usually situated near the central veins.

In both cases (inserts × 200) reticulin can be seen in proximity to the fatty cysts. (McGregor, × 50.)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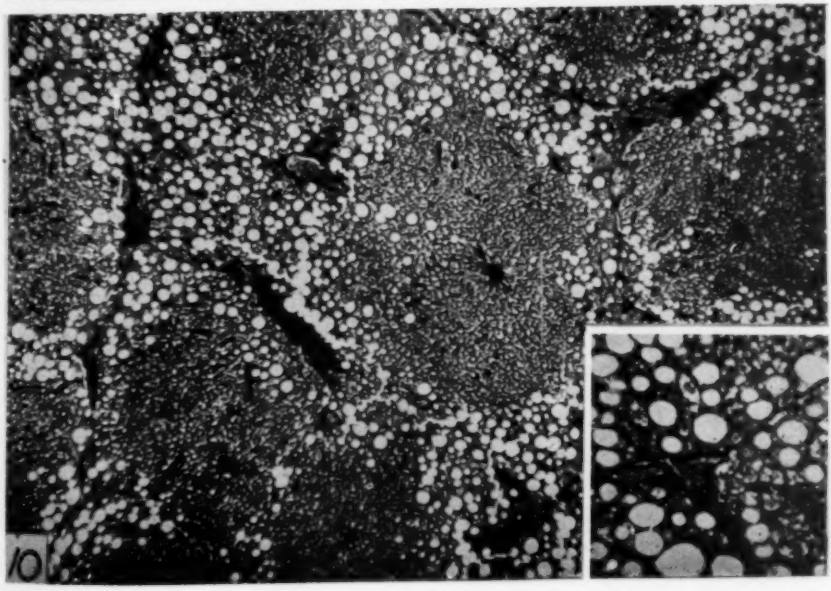
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PLATE 4

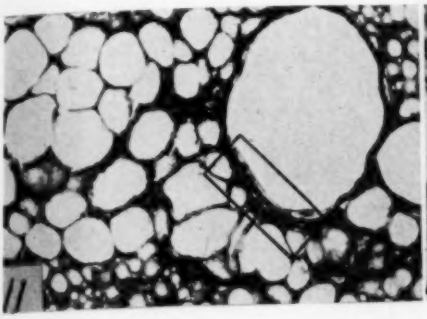
EXPLANATION OF FIGURES

11, 12 and 13 Higher magnification (× 160, × 1280, × 560) of sections from group fed hypolipotropic diet plus 0.1% thyroid. Note the cysts of varying size which with adjacent cells are encircled by reticulin fibres.

14 Gross specimen. Hypolipotropic diet with 0.02% propylthiouracil, 15 months. Similar appearance in controls after shorter period.

15 Hypolipotropic diet ad libitum for one year. Note the "fibrosis" and many fatty cysts. (× 160.)

16 Hypolipotropic diet with 0.02% propylthiouracil for 15 months. Note the "fibrosis" and many large fatty cysts. (× 160.)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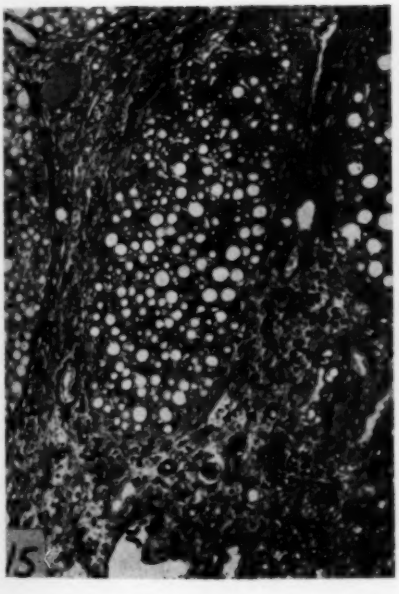
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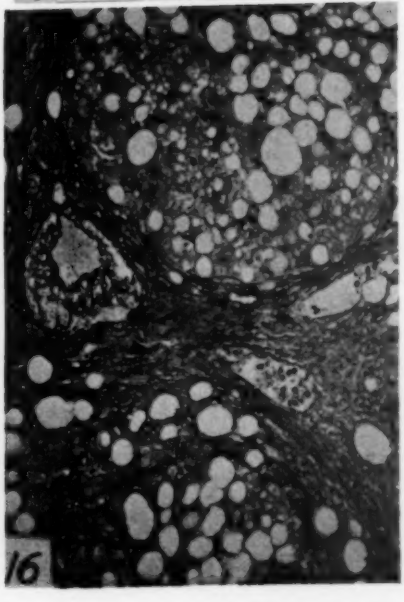
16

EFFECT OF THE TIME FACTOR AND CALORIE LEVEL ON NITROGEN UTILIZATION OF YOUNG WOMEN [1]

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(Received for publication March 10, 1951)

The importance of the time factor in feeding nutritive essentials was first shown by Berg and Rose ('29), who found that feeding rats half a daily allowance of tryptophan at intervals of 12 hours induced better growth than feeding the total daily allotment at one time. More recent work, which has been summarized and evaluated by Geiger ('50), shows that in laboratory animals both maintenance and growth require that all of the essential and the non-essential amino acids be present simultaneously as well as in adequate amounts.

Work in this laboratory has been concerned with the extent to which the time factor, in its relation to the supplying of food protein, affects the utilization of nitrogen by human subjects. Leverton and Gram ('49) have reported the nitrogen metabolism of 14 young women on a controlled adequate diet which included no animal protein in the breakfast, as compared with their nitrogen metabolism on the same diet when the breakfast included 240 gm of milk, although the total daily protein intake of approximately 63 gm remained unchanged. The mean daily nitrogen retention was — 0.03 gm

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when there was no milk in the breakfast, as compared with 0.96 gm when milk was included, and this difference in retention between the two periods was significant.

The present study was planned to determine whether, under similar conditions, the presence or absence of high quality protein in the noon meal would influence nitrogen metabolism, and the extent to which the level of intake of protein and calories affects the importance of the time factor.

TABLE 1

Plan of study

GROUP	DAILY PROTEIN INTAKE [1]	PERIOD A		PERIOD B		PERIOD C	
		Calories	Animal protein given in	Calories	Animal protein given in	Calories	Animal protein given in
	gm						
I			Breakfast		Breakfast		Breakfast
8 subjects	43	1,800	Dinner	2,400	Dinner	1,800	Lunch Dinner
II			Breakfast		Breakfast		Breakfast
8 subjects	63	1,800	Dinner	2,400	Dinner	1,800	Lunch Dinner

[1] Approximate — see table 2 for actual nitrogen content by analysis.

PROCEDURE

Sixteen young women served as subjects for this study for 54 days during the summer of 1949. The subjects ranged in age from 17 to 26 years, in height from 60 to 69 in., and in weight from 103 to 147 lb. They lived together in a small dormitory where the research staff supervised the entire program.

The master plan of the study is shown in table 1. Eight subjects were placed in each of the two groups designated as I and II. The only difference between group I and group II was the level of protein intake; group I received approximately 43 gm and group II approximately 63 gm daily. The chief daily sources of protein for group I were 480 gm milk and 60 gm lean meat as compared with 720 gm milk, 100 gm lean meat and 50 gm egg for group II.

For simplicity in referring to the levels of intake, "43" and "63" gm of protein will be used, because the actual nitrogen intakes with their slight variations are cumbersome in discussion.

The 54 days of the study were divided into three 18-day periods designated as A, B, and C.

During periods A and B there was no animal protein in the noon meal (except for milk solids in the commercial enriched white bread). During period C 240 gm of the milk served in the evening meal during periods A and B were transferred to the noon meal. The sources of animal protein in the evening meal were then 60 gm of meat for group I, and 100 gm of meat and 240 gm milk for group II. Thus, during period C animal protein was provided in each meal, although the total amount and kind of protein were the same as in the other periods.

Although the diets in periods A and B were identical in their distribution of protein, they differed in calorie value. In period A the calorie level was 1,800; in period B it was increased to 2,400 by the use of additional fat and sugar in each meal; and in period C it was identical to that in period A.

To facilitate rigid control of the amount and kind of food eaten by each group of subjects, a three-day quantitative diet including common foods was planned; this diet was repeated 18 times during the 54 days, or 6 times in each of the periods A, B, and C. The diets met the recommended allowances of the Food and Nutrition Board of the National Research Council ('48) for all nutrients except protein and calories.

The last 9 days of each of the three periods was a metabolism period. During this time weighed portions of all of the food eaten at each meal were combined into a composite. Stools for each subject for each period were marked with carmine. Food and fecal composites were made into hydrochloric acid digests before analysis for nitrogen by the macro-

Kjeldahl method. Urine collections for each subject for each 24-hour period were analyzed by the same method but without being ashed.

TABLE 2

Mean daily intake and excretion of nitrogen by all subjects in each group for three periods

		PERIOD A		PERIOD B		PERIOD C	
		Mean	S.D.[1]	Mean	S.D.[1]	Mean	S.D.[1]
Group I							
Intake	(gm)	6.84	0.19	6.99	0.33	7.12	0.17
Excretion	(gm)	7.48		6.40		7.05	
Urine	(gm)	6.77	0.80	5.56	0.80	6.27	0.83
Feces	(gm)	0.71	0.21	0.84	0.15	0.78	0.11
Retention	(gm)	— 0.64	0.56	0.59	0.59	0.07	0.64
Retention	(% of intake)	— 9.3		8.4		1.0	
Absorption	(% of intake)	89.4		87.9		89.1	
Group II							
Intake	(gm)	10.03	0.40	10.03	0.25	10.04[2]	0.24
Excretion	(gm)	9.85		9.06		9.84	
Urine	(gm)	9.10	0.96	8.23	0.83	9.08	0.92
Feces	(gm)	0.75	0.11	0.83	0.17	0.76	0.18
Retention	(gm)	0.18	0.59	0.97	0.48	0.20	0.63
Retention	(% of intake)	1.8		9.7		2.0	
Absorption	(% of intake)	93.0		91.7		92.4	

[1] Standard deviation.

[2] Five subjects only in group II, period C.

RESULTS

In table 2 are given the means and the standard deviations of the intake, excretion, and retention of nitrogen of the 8 subjects in each group for each 9-day metabolism period.

Effect of distribution among meals of protein from milk

The nitrogen metabolism of group I on a daily protein intake of approximately 43 gm was affected by the distribution of protein in the meals, while that of group II on the higher intake of 63 gm was not affected.

In period A, when all of the animal protein was in the morning and evening meals, group I had a mean percentage nitrogen retention of —9.3, and this rose to 1.0% in period C when the animal protein was redistributed so that milk protein was present in the noon meal. Although analysis of variance of the nitrogen intakes of each group did not indicate a significant difference during the three periods, the mean difference of 0.28 gm of nitrogen between the intakes of group I for periods A and C was significant at the 5% level ("t" = 3.24). The regression of intake on urinary excretion for periods A and C, however, was not significant ("b" = —0.56, "t" = 1.77), nor was the correlation of the intake and excretion ("r" = 0.15). Therefore, the variations in urinary nitrogen excretion were not related to variations in nitrogen intake. The mean urinary nitrogen excretion of period A, 6.77 gm, was significantly different ("t" = 3.68) from that of period C, 6.27 gm. The mean daily fecal excretion was 0.71 gm and 0.78 gm for periods A and C, respectively.

On the other hand, group II showed no difference in urinary nitrogen excretion between periods A and C. The mean daily urinary excretion was 9.10 and 9.08 gm and the percentage retention 1.8 and 2.0 for periods A and C, respectively.

Effect of calorie level

In period A, on 1,800 cal., the subjects in group I were not able to maintain nitrogen equilibrium on a daily intake of 6.84 gm of nitrogen but had a mean daily balance of — 0.64 gm. In period B the nitrogen intake was 6.99 gm, the calories were increased to 2,400, and the mean daily balance increased to 0.59 gm.

The increase in balance was a result of the highly significant ("t" = 12.16) decrease in mean daily urinary nitrogen from 6.77 gm in period A to 5.56 gm in period B.

In period A, on 1,800 cal., the subjects in group II had a daily intake of 10.03 gm of nitrogen and a mean daily balance of 0.18 gm. The balance increased to 0.97 gm in period B when the calories were increased to 2,400. Again the improved

balance was attributable largely to a decrease in the urinary nitrogen from 9.10 gm to 8.23 gm, a decrease which was highly significant ("t" = 10.63).

Thus, increasing the calories from 1,800 in period A to 2,400 in period B had a sparing action on nitrogen metabolism at both levels of intake, but the action was greater at the lower intake.

There was no change in body weight during period A but when the calories were increased from 1,800 to 2,400 during period B the mean total gain was 0.7 lb. for group I and 0.3 lb. for group II. When the calories were reduced to 1,800 in period C, the mean total loss was 1.8 lb. and 2.0 lb. for groups I and II, respectively.

DISCUSSION

When the daily protein intake was as generous as 63 gm, the presence or absence of animal protein in the form of milk at the noon meal did not affect the utilization of nitrogen as judged by urinary excretion. When the protein intake was only 43 gm, however, there was greater utilization of nitrogen when animal protein in the form of milk was present in the noon meal as compared with the period when there was no animal protein in the noon meal.

The data bear out the well-established nitrogen sparing action of calories from carbohydrate and fat. Under the controlled conditions of this study, increasing the calories from 1,800 to 2,400 accomplished more in conserving nitrogen at both levels of protein intake than redistributing the milk in the diet so that the noon meal furnished animal protein. Significantly, more nitrogen was stored at the lower intake of 43 gm protein with 2,400 Cal. than at the higher intake of 63 gm protein with 1,800 Cal., and the greatest storage of nitrogen occurred with an intake of 63 gm protein and 2,400 Cal.

Comparison of these results with those of an earlier study (Leverton and Gram, '49) indicates that lack of animal protein in the lunch was not as serious an impairment to nitrogen

utilization as its lack in the breakfast. A possible explanation is the difference in the number of hours which elapse between the morning and evening meal and between the evening meal and the noon meal on the next day.

The calories fed in the earlier study were slightly more generous than in the present study, ranging from 1,950 to 2,200. This may account in part for the lower mean daily urinary nitrogen excretion of 8.33 gm when the subjects in the first study were receiving animal protein in every meal, as compared with 9.08 gm for group II in period C of the present study under similar conditions of protein intake and distribution.

During the same time that this study was being conducted a group of 12 young women were on a weight reduction regimen and received a daily intake of approximately 63 gm of protein but only 1,200 calories during three 18-day periods (Leverton and Gram, '51). The regression of weight loss per period and the amount of overweight per period in nitrogen excretion were not significant; hence the nitrogen results may be compared with those of groups I and II reported here. The mean daily urinary nitrogen excretion was 8.94 gm when milk was in the noon meal, as compared with the 9.05 gm and 8.95 gm during two periods when there was no animal protein in the noon meal. Thus the distribution among the meals of the protein from milk did not affect the utilization of the nitrogen. The 1,200-calorie intake did not increase significantly the urinary excretion of nitrogen above the means of 9.10 and 9.08 gm for group II on 1,800 calories during periods A and C, respectively. These figures would indicate that the 1,800-calorie intake had no nitrogen sparing action above that of the 1,200-calorie intake when the protein level approximated 63 gm.

Because of convenience, milk was chosen as the source of animal protein to be redistributed between the noon and evening meals. The implications are, however, that other sources of high quality protein such as meat, fish, poultry, and eggs,

would have the same favorable effects under similar circumstances.

SUMMARY AND CONCLUSIONS

For 54 days two groups of 8 young women each were subjects for a study of the effect of the time factor and calorie level on nitrogen utilization. One group had a daily intake of approximately 43 gm of protein and the other of approximately 63 gm.

The effect on nitrogen excretion of the absence or presence of animal protein in the form of 240 gm milk in the noon meal, and the effect of a calorie level of 1,800 as compared with one of 2,400, were determined.

The group on the lower protein intake had a significantly lower urinary nitrogen excretion when animal protein in the form of milk was present in the noon meal than when it was absent. The group on the higher protein intake showed no difference in nitrogen excretion related to the presence or absence of animal protein in the noon meal.

There was a highly significant reduction in nitrogen excretion by both groups when the calories were increased from 1,800 to 2,400 and yet animal protein was absent from the noon meal. The nitrogen sparing action of the extra calories was greatest at the lower protein intake.

Thus the implications are that the lower the intake of protein and calories, the greater is the need for including high quality protein in each meal if nitrogen is to be well utilized.

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THE DIGESTIBILITY OF SIX TROPICAL FATS AS DETERMINED ON RATS [1]

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This is one of a series of studies on the nutritional value of plants and feedstuffs indigenous to Guatemala. Previous reports from this station — Squibb et al. ('50) and Squibb and Salazar ('51) — have been concerned with the evaluation of local plant and oil meals in baby-chick and swine diets. In the work presently reported several tropical fats were studied.

Markley and Jenkins ('49) have pointed out that tropical America has vast unexploited resources of fats and oils in the form of wild plants, and that it is possible to produce even greater quantities through the cultivation of indigenous or introduced species. These authors also stress the strategic importance of developing vegetable oil resources near the United States.

Over a period of years botanists have described most of the oil-bearing plants of these areas. Some chemical determinations of the properties of the fats and oils of these plants have been made and are reported in the literature but little work has been done on their biological value. This

[1] A contribution from the Instituto Agropecuario Nacional, a technical agricultural service organization for Guatemala, operated jointly by the Government of Guatemala and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. United States participation in this work was carried out as part of the Point IV program in Guatemala, administered by the Technical Cooperation Administration, U. S. Department of State.

[2] Animal husbandman, biochemist, and medical doctor in charge of nutrition laboratory, respectively — all of the Instituto Agropecuario Nacional.

article concerns the digestibility of 6 of these tropical fats — aceituno *(Simarouba glauca)*, cacao volador *(Virola guatemalensis)*, corozo (*Orbignya cohune* and species of genus *Scheelea*), morro *(Crescentia alata)*, tambor (*Omphalea oleifera)*, and sapayulo *(Calocarpum mammosum)* — as it was determined by studies on rats.

EXPERIMENTAL PROCEDURE AND RESULTS

The studies reported herein were conducted in a room of uniform temperature. The rats used were progeny of stock obtained from the Nutrition Laboratories of the Bureau of Animal Industry, United States Department of Agriculture. They were housed in individual all-wire cages with raised screen bottoms. Rations were fed ad libitum in special food cups equipped with circular disks punched with holes three-eighths of an inch in diameter. The disks rested on the feed in the cups and dropped with the feed as it was consumed. These disks served to reduce scattering of the feed and thus made it possible to keep more accurate consumption records. Water was available ad libitum in all cages.

Each experimental group consisted of 8 male and 8 female weanling rats. The rats were assigned so as to make the groups equal with respect to sex, age, and weight. They were weighed individually every week. The animals were fed the test diets for a period of 5 weeks. During the last 7 days of each experiment the feces of each group were collected and pooled and the total fat consumed by each group during this period recorded. These data were used to calculate the digestion coefficients. The analyses of the feces, the correction for metabolic fat, and the calculation of the digestion coefficients were made according to the method of Augur et al. ('47).

The low fat basal ration consisted of "vitamin-test" casein, 20 gm; sucrose, 76 gm; U.S.P. no. XII salt mixture, 4 gm; and the following vitamin supplements per 100 gm ration: 50 mg carrot oil (25,000 U.S.P. units per gram); delsterol, 20 mg; choline, 125 mg; thiamine, 0.2 mg; riboflavin, 0.35 mg;

pantothenic acid, 1.20 mg; pyridoxine, 0.35 mg; and nicotinic acid, 1.5 mg.

The correction for metabolic fat was found to be 71 mg per gram of dried stool. This factor is slightly higher than that (65 mg per gram of dry stool) found by Deuel et al. ('48).

TABLE 1

Some chemical and physical data on the fats tested

COMMON NAME	SCIENTIFIC NAME	IODINE NO.	SAPON. NO.	FREE FATTY ACIDS	MELTING POINT	SOURCE OF DATA [1]
				gm	*°C.*	
Aceituno [2]	*Simarouba glauca*	56.4	193.5	14.0		IAN
Cacao volador	*Virola guatemalensis*	10.5–18	218–228		40–47	Jam.
		16.1	241	0.63	41.5	IAN
Corozo	*Orbignya cohune* and species of genus *Scheelea*	9.8	251	0.9		Jam.
		53.3	230	0.23		IAN
Morro	*Crescentia alata*	87.6	195.4	1.46		D.S.
		90.0	190.0	1.10		Lewy
		85.0	193.5	0.23		IAN
Nieve [3]	*Simarouba glauca*	53.8	191.0	0.06	25.0	D.S.
Tambor	*Omphalea oleifera*	112.8	194.2	10.02		D.S.
		103.0	196.2			IAN
Sapayulo	*Calocarpum mammosum*	65.3	192.0	3.75		D.S.
		62.2	193.5	0.34		IAN

[1] D.S.: H. De Sola and Sons Co., El Salvador (unpublished data, '50).
IAN: Instituto Agropecuario Nacional.
Jam.: Jamieson ('43).
Lewy; Lewy ('48).
[2] Unrefined.
[3] Refined, commercial product.

Six different fats were tested as crude and partially refined products at levels indicated in table 2, replacing a portion of the sugar of the basal ration. The partial refining consisted of treating the fats with an amount of sodium hydroxide calculated to neutralize the free fatty acids, heating to the break point and separating the resulting soap.

The fats were then washed until free of alkali, and dried. One fat, cacao volador, was heated and treated with 10% HCl to aid in removing resins; this preparation was then refined by the method previously described.

TABLE 2

Summary table of digestibility of several crude extracted and partially refined fats of tropical origin

TRIAL NO.	FAT	LEVEL TESTED	FAT CONSUMED	FAT IN FECES				COEF. DIG.
				Neutral	Soap	Total	Total [1]	
		%	gm	gm	gm	gm	gm	%
1	Nieve	10	69.7	3.28	7.41	10.69	9.18	86.8
	Corozo	10	63.8	1.98	1.82	3.80	2.83	95.6
2	Tambor	10	70.8	1.83	2.20	4.03	2.91	95.9
	Morro	10	71.6	2.14	1.63	3.77	2.57	96.4
	Mazola	10	66.6	1.98	0.69	2.67	1.69	97.4
3	Sapayulo	15	90.5	7.15	6.07	13.22	11.39	87.4
	Aceituno	15	65.1	1.70	3.57	5.27	4.21	93.5
	Cacao volador	15	47.1	4.15	1.26	5.41	4.72	89.9
4	Corozo [2]	10	37.8	0.98	0.84	1.82	1.13	97.0
	Morro [2]	10	39.3	1.61	0.66	2.27	1.40	96.4
	Tambor [2]	10	37.5	1.38	1.53	2.91	2.07	94.5
5	Sapayulo [2]	15	105.0	6.48	3.22	9.70	8.17	92.2
	Cacao volador [2]	15	89.3	2.80	0.97	3.77	2.76	96.9

[1] Corrected for metabolic fat.

[2] Refined fats.

The scientific names of the plants from which the fats were obtained and some chemical data related to the fats tested are presented in table 1. The amount of neutral fats and soaps excreted in the feces and the digestion coefficients are presented in table 2.

DISCUSSION

Since the effects of feeding most of the fats tested were biologically unknown, they were fed over a 35-day period both as crude and partially refined products so that any possible toxic effects could be observed.

All groups of rats grew fairly uniformly with the exception of the group fed the unrefined cacao volador fat. Some depression of growth was noted in this group. This depression was not observed in the group fed the refined fat.

As may be seen in table 2, the coefficients of digestibility of the fats compared favorably with that of corn oil.[3] These digestion coefficients were not markedly improved by refining; exceptions were the cacao volador fat and, to a lesser extent, the sapayulo oil. For an unknown reason the coefficient of digestibility of Nieve fat, a commercial product prepared from aceituno fat, was lower than that of the crude product.

The limited data presented herein support the statement by Markley and Jenkins ('49) that these tropical areas contain valuable fat resources. Aceituno fat, for example, has been found to resemble chemically a composite sample of some 5 leading vegetable shortenings used in the U. S.[4] A commercial company has distributed more than one-half million trees as the start of an intensive program to increase the production of this fat. Other fats may be equally important. Markley ('50) has pointed out that the seeds or fat of cacao volador could become an important item of export to the United States, where the fat would be useful in the confectionery and baking industries as an enrobing fat.

Corozo oil has not been fully exploited. Corozo palms are plentiful in many areas of tropical America. Rothe ('50) has counted as many as 1,300 palms per acre in the Lake Izabal area of Guatemala. Corozo oil at present is being used principally in the soap industry, for the flavor of the oil has a tendency to change.

SUMMARY

For 6 partially refined fats of tropical origin, some chemical data were obtained and digestion coefficients determined with rats. The coefficients of digestibility for the 6 were as fol-

[3] Mazola.

[4] According to unpublished information from the H. De Sola and Sons Company of El Salvador.

lows: Corozo (*Orbignya cohune*, and species of the genus *Scheelea*), 97.0; morro (*Crescentia alata*), 96.4; tambor (*Omphalea oleifera*), 94.5; sapayulo (*Calocarpum mammosum*), 92.2; cacao volador (*Virola guatemalensis*), 96.9; and unrefined aceituno fat (*Simarouba glauca*), 93.5.

The data indicate that there are many valuable fats in tropical America which are of strategic importance. These essential vegetable oil resources should be developed through the cultivation of indigenous or introduced oil plants.

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A STUDY OF THE ENDOGENOUS NITROGEN OUTPUT OF COLLEGE WOMEN, WITH PARTICULAR REFERENCE TO USE OF THE CREATININE OUTPUT IN THE CALCULATION OF THE BIOLOGICAL VALUES OF THE PROTEIN OF EGG AND OF SUNFLOWER SEED FLOUR

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ONE FIGURE

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In assessing the comparative nutritive value of proteins in adult nutrition by the Thomas ('09) method, reproducible endogenous urinary nitrogen values are of fundamental importance. Murlin et al. ('46) have referred to attempts by several authors to obtain reliable estimates of endogenous nitrogen values in considering some of the factors which affect determined values.

The present study reports the nitrogen excretion of 25 women on low-nitrogen diets for periods of 14 to 15 days. The urinary nitrogen in this instance is considered as endogenous and the fecal portion as metabolic. In addition, an investigation has been made of the possibility of estimating endogenous nitrogen from numerical factors related to body size, basal metabolism or urinary creatinine nitrogen. The possible relationship between metabolic fecal nitrogen and

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dry matter consumed is considered. The observed and estimated endogenous and metabolic values are applied to the calculation of the biological values of whole egg and sunflower seed flour.

EXPERIMENTAL PROCEDURE

Subjects

Twenty-five college women served as experimental subjects over a period of three years. They ranged in age from 19 to 30 years. Individual data for age, height, weight, surface area and basal metabolism are listed in table 2. The first group of 10 subjects (group 1) participated in the initial study, 1945–1946; a second group of 10 (group 2) were in the 1946–1947 study; and 5 more subjects (group 3) participated during 1947–1948. Subject B, who took part in both the 1945–1946 and 1946–1947 studies, was the only individual who participated for more than one year.

Diet

The low-nitrogen diet used throughout these studies (described in detail by Bricker et al., '49) was designed to supply adequate minerals, vitamins, and calories and to be as low in nitrogen as possible (about 0.2 gm nitrogen per day). The chief dietary item consisted of cookies containing cornstarch, sucrose, washed butterfat, agar, corn oil, NaCl, baking powder, and salt mixture. One hundred milliliters of lemon juice increased the palatability of the diet by adding variety of flavor and by furnishing a vehicle for part of the sugar. Sugar and butterfat, with one-third of the total calories as fat, were used to adjust the total calories. Usually 45 cal. per kilogram of body weight were fed, but this amount was increased or decreased as needed to maintain constant body weight (see table 2 for individual values). Vitamins [2] were supplied by two tablets daily, one in the morning and one in the evening.

[2] Stuart formula tablets, Stuart Laboratories, Pasadena, California.

Together these furnished 4.0 mg thiamine chloride, 4.0 mg riboflavin, 30.0 mg nicotinamide, 5.0 mg calcium pantothenate, 0.2 mg pyridoxine, 100 mg ascorbic acid, 4.0 mg mixed tocopherols, 5,000 U.S.P. units vitamin A, and 800 U.S.P. units vitamin D daily.

When eggs or sunflower seed flour were used as protein sources, total calories and quantities of fat were adjusted to allow for the contributions of these substances by the test foods. No adjustment was made for the extra vitamins and minerals added thereby. Daily protein supplements were divided equally among three meals. When eggs supplied the protein, they were used as whole eggs [3] beaten together in daily lots in order to insure uniform sampling, cooked at a low temperature over hot water, and weighed in individual portions after cooking. The sunflower seed meal [4] was incorporated into the cornstarch cookies and baked, with time and temperature controlled.

Dietary regime

The diet periods, listed in table 1 in the order in which they occurred, were selected from a series of studies on low protein diets which each year extended from September to June.

Analytical methods

The methods of collecting, preparing, and analyzing the samples of food and excreta for nitrogen have been described previously (Bricker et al., '49). Urinary nitrogen was determined daily and fecal values were obtained on 5-day pools. Analyses for creatinine were made daily in all low-nitrogen periods and periodically during the other periods by the Folin ('14) method as modified by Peters ('42) for use with the photoelectric colorimeter.

[3] Eggs were obtained from the University Poultry Farms.

[4] Supplied by the Viobin Corporation, Monticello, Illinois. Obtained in three lots, one each year. The method of preparation and the composition of the flour are described in detail in the article of Mitchell et al. ('45).

Selection of days on which urinary values were typical of a given dietary regime

During low-nitrogen periods the urinary values for the last 5 days were averaged to give an endogenous value. When egg or sunflower seed flour was included, an average was made of the daily urinary values occurring after the initial rapid changes had occurred (indicative of adjustment to the diet in question).

TABLE 1

Experimental periods

PERIOD	DIET GROUP	NO. OF SUBJECTS	DATE	DIET	NITROGEN CONTENT
					gm/day
1a	1	10	Jan. 4–18, 1946	Low-nitrogen	0.16
1b	1	9	April 26–May 14, 1946	Sunflower, 30 gm	2.81
2a	2	10	Dec. 3–16, 1946	Low-nitrogen	0.21
2b	2	10	Feb. 28–Mar. 6, 1947	Egg, 80 gm	2.02
2c	2	10	Mar. 7–16, 1947	Low-nitrogen	0.23
2d	2	9	May 10–29, 1947	Sunflower, 30 gm	2.94
3a	3	5	Dec. 2–15, 1947	Low-nitrogen	0.22
3b	3	5	Feb. 22–Mar. 4, 1948	Sunflower, 35 gm	3.60
3c	3	4	May 17–30, 1948	Low-nitrogen	0.25

PRESENTATION OF DATA

As already indicated, individual data for age, height, weight, surface area and basal metabolism appear in table 2. Nitrogen excretion data for 25 subjects on the low-nitrogen diet (periods 1a, 2a, 3a) are presented in table 3, which lists the urinary endogenous and creatinine nitrogen in milligrams per day, per kilogram, per square meter of surface area, and per basal calorie; the per cent of endogenous nitrogen appearing as creatinine nitrogen; and the metabolic fecal nitrogen, expressed in milligrams per day and milligrams per gram of dry matter consumed. Table 4 shows endogenous nitro-

TABLE 2

Age, body size, basal metabolism and per kilogram allowance of calories for 25 subjects in a study of protein metabolism

	SUBJECT	AGE	BODY WEIGHT	STANDING HEIGHT	SURFACE AREA [1]	BASAL METABOLISM [2]	CALORIC ALLOWANCE
		years	*kg*	*cm*	m^2	*cal./day*	*cal./kg*
Group 1	A	24	46	164	1.48	1,257	45
	B	19	67	171	1.78	1,486	45
	C	24	54	158	1.55	1,197	45
	D	30	58	167	1.65	1,245	45 [3]
	F	25	76	180	1.96	1,469	45 [3]
	G	22	48	157	1.45	1,010	45
	H	22	49	163	1.52	1,200	50
	I	24	66	167	1.74	1,249	45
	J	26	70	166	1.78	1,248	45 [3]
	K	22	65	173	1.78	1,343	45
Group 2	B	20	60	171	1.70	1,220	40 [3]
	L	21	50	157	1.48	1,149	45
	M	20	56	157	1.55	1,170	45
	N	23	60	162	1.64	1,433	45
	O	24	68	167	1.76	1,245	40
	P	19	64	161	1.67	1,276	45
	R	19	51	157	1.49	1,389	45
	S	26	56	178	1.70	1,311	45
	T	23	70	158	1.72	1,294	40
	U	22	45	152	1.38	1,232	50 [3]
Group 3	Bb	20	67	162	1.65	1,351	42
	Cc	26	58	178	1.72	1,271	45
	Dd	22	57	170	1.72	1,276	48
	Ff	23	50	158	1.44	1,201	45
	Gg	21	48	159	1.43	1,404	45

[1] Estimated by the DuBois-DuBois formula:

$$SA_{sq.m} = 71.84 \times Wt._{kg}^{0.425} \times Ht._{cm}^{0.725}.$$

[2] Determined by oxygen consumption measured by the McKesson Waterless Metabolor.

[3] Shows a deviation from indicated calorie allowance. These exceptions are: subjects D, F, and J, who received 40 cal. per kilogram in period 1b. (see dietary regime, table 1) and subjects B and U, who received 35 and 45 cal. respectively in period 2d.

gen, urinary creatinine nitrogen, and metabolic fecal nitrogen values for 14 subjects on low-nitrogen diets in periods 2c and 3c. Figure 1 shows daily endogenous urinary values, together with ratios of creatinine nitrogen to endogenous nitrogen values for each of the subjects of group 1. Data on the biological value and true digestibility of whole egg and sunflower seed flour are presented in table 5.

DISCUSSION OF RESULTS

Maintenance of body weight on low-nitrogen diets

Nude weights of the subjects were recorded each morning before breakfast. With one exception (subject B, period 2d), there was no upward or downward trend in weight when 40 to 50 cal. per kilogram of body weight were fed to each of the subjects. The constancy of weight indicates that these caloric intakes are capable of preventing loss of body mass when the diet is practically devoid of protein. The use of body protein for energy purposes was undoubtedly at a minimum.

Length of period for endogenous nitrogen estimation

In reaching a decision concerning the length of period required for arriving at endogenous values, numerical values as well as plotted data for urinary nitrogen and the ratios of creatinine nitrogen to endogenous nitrogen were examined. The method of plotting is illustrated in figure 1 for group 1. It can be observed that in no instance before the 4th day after the beginning of the low-nitrogen diet would one have been assured of a plateau in urinary nitrogen excretion or in the $\frac{CN}{EN}$ ratio, and in one instance (subject H) as many as 10 days would have been required. A 9-day preliminary period would have permitted satisfactory adjustment for most of the subjects. The decision was made, therefore, to use an average of the last 5 days in a 14-day period routinely, since in our experience this was the minimum time

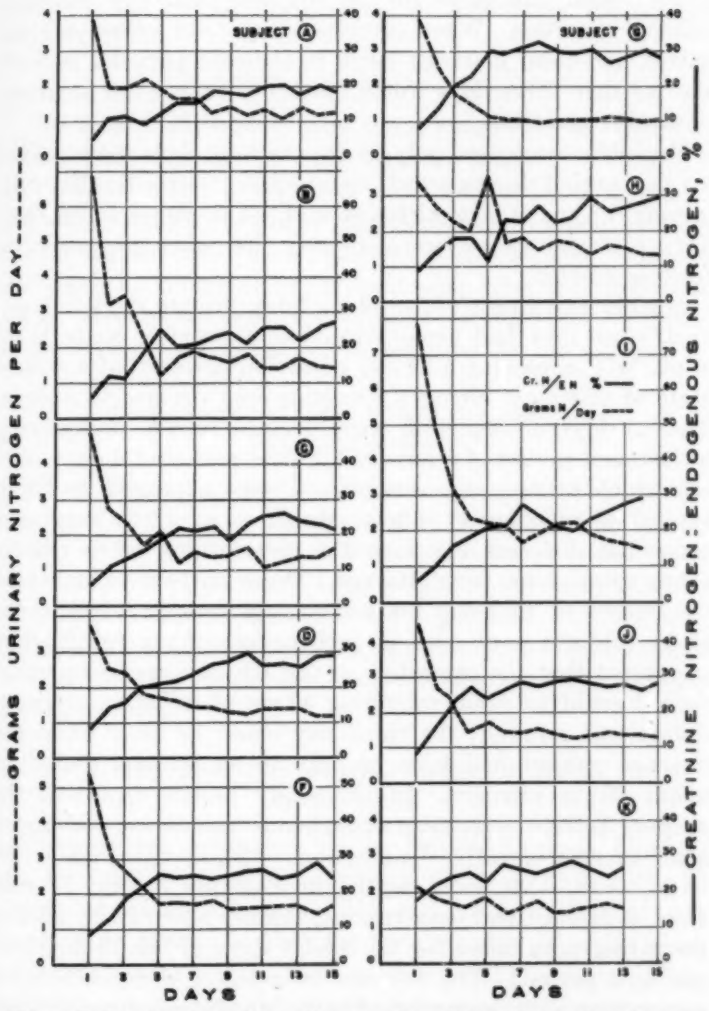

Fig. 1 Daily values for urinary nitrogen and creatinine nitrogen to endogenous nitrogen ratios, plotted for the 10 subjects of group 1 on a low-nitrogen diet for 13 to 15 days.

which would guarantee for all subjects relatively constant urinary excretion. When corresponding fecal values are required and fecal markers serve to indicate periods, periods shorter than three days would markedly reduce the accuracy of stool separations.

Period 2c comprised only 10 days *in toto,* since it succeeded a 7-day period during which the subjects received a diet containing 1.8 gm of egg nitrogen and, as a consequence, urinary nitrogen values were already low when low-nitrogen feeding started.

Mueller and Cox ('47) used a 12-day protein depletion period, since they had demonstrated in an earlier study (Cox et al., '47) employing a 21-day depletion period that a steady state of minimum nitrogen excretion was reached in 8 days. The 12 days of depletion were divided into a preliminary adjustment period of 8 days and a final period of 4 days during which urinary nitrogen values were averaged as being typical of endogenous values. Murlin et al. ('46) suggest a somewhat different criterion for determining when endogenous values have been attained. These workers summarized the results of 12 4-day low-nitrogen periods in which three to 11 subjects were used in each period. As a result, they suggested that the excretion on the 4th day of a no-protein period could be used, providing 40 gm of negative nitrogen deficit had accrued; the third day could be used when the average subject had been brought to an accrued deficit of about 60 gm nitrogen. In the study herein reported, the authors believe acceptable endogenous values were obtained after accrued negative balances of — 21, — 27, — 22, — 20, — 24, — 18, —25, — 32, — 21, and — 24 gm for the 10 subjects in group 1 had been reached (values obtained by adding the nitrogen balances for the first 9 days of the 15-day low-nitrogen period). The subjects in group 1 were chosen for comparison with those studied in the Murlin experiments since they began the low-nitrogen diet immediately following a 15-day period of unrestricted protein intake. This should remove the necessity for including any negative balances

prior to the low-nitrogen period in estimating the accrued balance.

Estimated daily endogenous losses

The mean daily endogenous urinary excretion was 1,454 mg (S.E., 30.8) for the 25 subjects (see table 3). Values of 1,910, 1,510, 1,310, 1,800, 1,570, and 1,240 mg for 6 women reported by Hawley et al. ('48) include two which are definitely higher than those reported in this paper. Values of 1,919 and 2,131 mg for two women were reported by Bricker ('45). Most of the scattered values available for men appear to be higher than those herein reported for 25 women.

Endogenous nitrogen values per kilogram of body weight

These values for the 25 subjects appear in table 3. The average excretion per kilogram of body weight was 25.2 mg (S.E., 0.65). Murlin et al. ('46) observed an average value of 38.9 mg per kilogram of body weight for 85 cases in which data on men and women were not separated. This average, reported for the third day of "no-protein" feeding, decreased to 31.8 for 22 of the same cases (14 men) on the 4th day of "no-protein."

Endogenous nitrogen per square meter of surface area

These values, estimated by the DuBois formula (DuBois and DuBois, '16), averaged 898 mg (S.E., 17.0) for the 25 women. Average values of 1,173 and 1,101 for 5 men were reported by Murlin et al. ('46) for duplicate determinations made one month apart. Hawley et al. ('48) presented data for surface area and endogenous nitrogen for 6 women and 7 men. When these data were expressed per square meter of surface area, the average values were 1,138 and 933 for men and women, respectively.

TABLE 3

The endogenous urinary nitrogen, urinary creatinine nitrogen and metabolic fecal nitrogen excretions of subjects on nitrogen-low (0.2 gm daily) diets

	SUBJECT	PERIOD	ENDOGENOUS URINARY NITROGEN				$\left[\frac{CN}{EN}\right]^1$	CREATININE NITROGEN			METABOLIC FECAL NITROGEN	
			Per day	Per kg	Per m²	Per basal calorie		Per day	Per m²	Per basal calorie	Per day	Per gm dry matter [2]
			mg	*mg*	*mg*	*mg*	%	*mg*	*mg*	*mg*	*mg*	*mg*
Group 1	A	1a	1,320	28.6	892	1.05	20.0	264	178	0.210	400	0.830
	B	1a	1,525	22.8	857	1.03	25.2	384	216	0.258	429	0.663
	C	1a	1,304	23.9	841	1.09	24.1	314	203	0.262	437	0.761
	D	1a	1,309	22.5	793	1.05	27.5	360	218	0.289	478	0.846
	F	1a	1,618	21.3	826	1.10	26.0	420	214	0.286	556	0.772
	G	1a	1,064	22.0	734	1.05	28.9	307	212	0.304	442	0.917
	H	1a	1,389	28.1	914	1.16	27.6	384	253	0.320	619	1.146
	I	1a	1,580	23.9	908	1.26	27.5	434	249	0.347	643	0.997
	J	1a	1,330	19.1	747	1.07	27.4	364	204	0.292	550	0.827
	K	1a	1,503	23.1	844	1.12	26.7	402	226	0.299	458	0.709
Group 2	B	2a	1,635	27.1	967	1.34	23.2	378	224	0.310	554	0.947
	L	2a	1,461	29.2	994	1.27	23.8	347	236	0.302	404	0.821
	M	2a	1,509	26.8	967	1.29	22.6	340	218	0.291	395	0.719
	N	2a	1,689	28.3	1,056	1.18	22.9	386	241	0.269	470	0.817
	O	2a	1,563	22.9	893	1.26	24.6	379	217	0.304	490	0.821
	P	2a	1,505	23.7	923	1.18	26.8	399	245	0.313	470	0.793
	R	2a	1,272	24.8	859	0.92	24.3	308	208	0.222	387	0.757
	S	2a	1,524	27.4	907	1.16	24.6	371	221	0.283	508	0.912
	T	2a	1,397	20.0	827	1.08	25.0	348	206	0.269	602	1.019
	U	2a	1,320	29.1	957	1.07	24.5	323	234	0.262	468	0.912
Group 3	Bb	3a	1,594	23.8	966	1.18	25.0	397	241	0.294	521	0.871
	Cc	3a	1,402	24.2	815	1.10	26.5	369	215	0.290	712	1.228
	Dd	3a	1,734	30.4	1,008	1.36	23.8	411	239	0.322	536	0.901
	Ff	3a	1,322	26.6	918	1.10	26.3	344	239	0.286	527	1.093
	Gg	3a	1,490	31.1	1,042	1.06	22.8	336	235	0.239	489	1.036
Mean			1,454	25.2	898	1.14	25.1	363	224	0.285	502	0.885
Standard deviation			153.8	3.27	85.0	0.107	2.00	40.1	18.0	0.0311	82.1	0.1400
Standard error			30.8	0.65	17.0	0.021	.40	8.0	3.5	0.0062	16.4	0.0280
Coefficient of variation			10.6%	13.0%	9.5%	9.4%	8.1%	11.1%	7.8%	10.9%	16.4%	15.8%

[1] Urinary creatinine nitrogen ÷ endogenous urinary nitrogen × 100.
[2] Dry matter consumed.

Endogenous nitrogen per basal calorie

These data averaged 1.14 mg (S.E., 0.021) for the 25 female subjects herein reported on, a value in close agreement with the average value of 1.19 mg for 6 women reported by Hawley et al. ('48). In the same paper a value of 1.32 was reported for 7 men. Smuts ('35), on the basis of extensive investigations of this relationship in mice, rats, guinea pigs, rabbits, and pigs, concluded that there is "a close relationship between the total nitrogen excretion of warm-blooded animals varying widely in size and their basal metabolism, such that 2 mg of nitrogen are lost to the body daily for every calorie of basal heat." On the basis of published data on two adult male subjects (Smith, '26; Deuel et al., '28), Smuts further concluded that "the relationship appears to apply also to the human species." Examination of the data cited by Smuts in support of this last statement indicates that neither subject was maintaining body weight, and hence the endogenous nitrogen loss may have been augmented to some degree by undue breakdown of body tissue. In the latter portion of the studies, when body weight was constant and the endogenous nitrogen lower, the ratio of endogenous nitrogen to basal calories was considerably below 2.0. The ratio for Smith's subject on the last day of a 24-day period was 1.0, and that of Deuel's during the last 7 days of a 30-day period was 1.5.

A value of 1.425 mg of endogenous nitrogen per basal calorie, derived from data for 9 subjects and reported earlier from this laboratory (Bricker et al., '45), was higher than those herein reported. The use of three- or 4-day periods for endogenous nitrogen determinations, together with lower caloric intakes, may well account for the higher values obtained in the earlier study.

The possibility of sex as a causative agent for differences in the ratio reported by Smuts and those of the present investigation should be considered. Only one paper was found in which data were reported for men and women studied under similar conditions. In this instance (Hawley et al.,

'48), the average of 1.19 for women, though lower than that of 1.32 for men, was not significantly so, due to overlapping of data. Data reported by Palmer, Means and Gamble ('14) on basal metabolism-to-creatinine relationships have a bearing on this question. An average ratio of 1.26 Cal./mg reported for 9 women is significantly higher than the average of 0.98 for 8 men. Since urinary creatinine and endogenous nitrogen have been shown to be closely related, it would be expected that the sex difference shown above would extend to the endogenous nitrogen-to-basal metabolism relationship.

Endogenous nitrogen and creatinine

The nitrogen excreted as creatinine averaged 363 mg per day (S.E., 8.0) for the 25 subjects, as is seen in table 3. When the creatinine nitrogen was related to surface area, an average value of 224 mg per square meter (S.E., 3.5) was obtained.

Expressed in terms of basal calories, the creatinine nitrogen averaged 0.285 mg per basal calorie (S.E., 0.0062) or, expressed conversely, 1.30 cal. per milligram of creatinine. Using this mode of expression, Palmer, Means and Gamble ('14) and Wang et al. ('30) cite values for women of 1.26 and 1.05, respectively.

When the creatinine nitrogen was expressed as a per cent of the total urinary nitrogen in the low-nitrogen period, relatively constant percentages were obtained after the initial adjustment period (see fig. 1 for group 1). For the 25 cases the mean value after adjustment was 25.1 (S.E., 0.40; see table 3). This mean is not too different from certain values recorded by various investigators for individual men subsisting on nitrogen-low diets. Graham and Poulton ('12) reported a ratio of 26.6 for the 9th day of an experiment in which the subject was given 0.91 gm nitrogen per day. Robison ('22) had two subjects whose values were 20.2 and 23.3 each on the 7th day while taking a diet containing 0.33 gm nitrogen daily. Smith ('26), in a study of one subject on 0.33 gm nitrogen daily, recorded 35.4 on the 24th day. Deuel et al. ('28)

observed 26.7 for one subject who had consumed 0.26 gm nitrogen daily for 30 days. Murlin et al. ('48) reported 25.3% as a mean of 39 determinations on subjects on "no-protein" diets. The number of subjects and the division among men and women are not indicated. These workers further state, "It would seem that the biological value of any mixed diet could be obtained approximately by feeding it at a level low enough to obtain a minus N balance and determining the creatinine nitrogen percentage. The possibility is now under investigation."

In the present study, the coefficient of variation was used as a device for comparing the desirability of the above 4 methods of expressing the endogenous nitrogen excretions of individuals. The coefficient of variation for the data on endogenous nitrogen per person is 10.6 (see table 3). Coefficients of 13.0, 9.5, and 9.4 were obtained when the individual data were expressed per kilogram of body weight, per square meter of surface area, and per basal calorie, respectively. Although the values of 9.5 and 9.4 were lower numerically than the other two values, there was no statistically significant [5] difference in any of the possible comparisons. Expression of the per person data on the basis of body weight tended to increase the variation, as is shown by the change in the coefficient from 10.6 to 13.0. The authors were surprised to find such a low coefficient for the per person data. It would seem probable that the variation of endogenous nitrogen values on a per person basis would increase in a population including both men and women and a range of ages.

The coefficient of variation for creatinine nitrogen expressed as a per cent of total endogenous nitrogen was 8.1 for the 25 cases, which is a lower value than any of the

[5] The difference between two coefficients of variation was considered significant if it was two or more times the standard error of the difference between the two coefficients of variation. The following formulae were used:

$$SD_{cv} = \frac{CV}{\sqrt{2}}\sqrt{1 + 2(CV)^2}, \quad SE_d = \sqrt{\frac{(SD_{cv_1})^2}{N_1} + \frac{(SD_{cv_2})^2}{N_2}},$$

where SD refers to standard deviation, CV to coefficient of variation and SE_d to the standard error of the difference.

others derived for endogenous nitrogen, although not significantly so except in the comparison with milligrams per kilogram body weight. One might then predict net nitrogen needs more accurately by use of creatinine nitrogen data than by means of either surface area as estimated by the DuBois formula (DuBois and DuBois, '16) or basal calories. It appears that a fairly reliable estimate of endogenous nitrogen could be obtained by multiplying the creatinine nitrogen excretion on a creatinine-low diet by 3.984.[6]

Metabolic nitrogen

The amount of nitrogen appearing in the feces on the low-protein diets, as listed in table 3 for each of the subjects, averaged 502 mg nitrogen per person per day (S.E., 16.4). The individual values showed considerable variation, as evidenced by the coefficient of variation of 16.4%. Mitchell ('26) has reported that "the most important factor determining the amount of metabolic nitrogen in the feces is the amount of dry matter consumed." When values were expressed in this manner in the present study, the mean was 0.885 mg (S.E., 0.028) of fecal nitrogen per gram of dry matter consumed. Values of 0.91 and 1.14 mg per gram dry matter consumed were also reported for two women by Bricker et al. ('45) and are within the range of those in this paper. The metabolic fecal values expressed as a per cent of the endogenous nitrogen averaged 34.9 (S.E., 1.30).

On the basis of the above information a quick estimate of metabolic nitrogen could be obtained by multiplying the grams of dry matter consumed by 0.88 mg in cases where the composition of the test diet is similar to that of the diet used in this study.

Duplicate determinations of endogenous nitrogen

These determinations were made for 14 subjects in groups 2 and 3, three to 5 months after the initial test. During the

[6] If, as shown in table 3, 25.1% of total endogenous nitrogen is excreted as creatinine nitrogen, the endogenous nitrogen $= \frac{1}{0.251}$ or $3.984 \times$ creatinine nitrogen.

interim the subjects had been on low-protein diets (1.4 to 4.0 gm N daily) almost continuously. A comparison of these data (table 4) with those of the initial test (table 3) reveals that all subjects except R showed higher endogenous urinary excretions in the first than in the second determination.

From unpublished data in this laboratory, it appears that the tendency to lower endogenous values on the occasion of

TABLE 4

The endogenous urinary nitrogen, urinary creatinine nitrogen and metabolic fecal nitrogen excretions of subjects on nitrogen-low (0.2 gm daily) diets during periods 2c and 3c

	SUBJECT	PERIOD	DAILY NITROGEN EXCRETION		
			Endogenous	Creatinine	Fecal
			mg	*mg*	*mg*
Group 2	B	2c	1,407	330	571
	L	2c	1,203	322	444
	M	2c	1,217	334	406
	N	2c	1,370	347	505
	O	2c	1,385	358	409
	P	2c	1,378	387	361
	R	2c	1,372	306	322
	S	2c	1,272	353	421
	T	2c	1,336	320	526
	U	2c	1,210	316	521
Group 3	Bb	3c	1,386	354	458
	Cc	3c	1,369	337	581
	Ff	3c	1,176	293	370
	Gg	3c	1,114	305	415

the second determination may result from the adapation of individuals to continued feeding of low-protein diets. Murlin et al. ('46) call attention to a tendency for lower urinary nitrogen in the later periods of series in which more than one "no-protein" period is used, and discuss as possible influencing factors "the nature of the food protein which preceded the no-protein, the level at which it was fed, and the length of time."

Coincident with the lowered endogenous nitrogen noted in the second test was a fall in the creatinine excretion for every subject. This tended to maintain a constant $\frac{CN}{EN}$ ratio. The metabolic nitrogen values showed no consistent change during the second test; 9 decreased and 5 increased.

Biological values of sunflower seed flour and whole egg

Because of the high protein content of sunflower seed flour, the authors were interested in testing its biological value for humans. It had further been shown in the Foods Research Laboratory in this Department that the replacement of as much as 20% of the wheat flour in certain baked goods with sunflower seed flour resulted in palatable products. Tests of sunflower seed flour conducted on 23 subjects (groups 1, 2, and 3) according to the Thomas ('09) method showed values of 61.2 (S.E., 1.71) and 89.9 (S.E., 0.86) for biological value and true digestibility. Mitchell et al. ('45) reported average values of 64.5 for biological value and 94.3 for true digestibilty, using 10 rats.

It is of interest to compare determined values for biological value with those estimated by use of the factors for endogenous and metabolic nitrogen discussed in the earlier part of this paper. Since creatinine values during the time of sunflower feeding were available for groups 2 and 3 only, these data were used in the comparison. As shown in table 5, the determined biological values for these 14 subjects averaged 60.4 (S.E., 2.28), whereas the calculated values averaged 61.8 (S.E., 2.94). The calculated biological value was computed by use of endogenous urinary nitrogen values as estimated from the creatinine excretion during the sunflower periods, together with determined metabolic nitrogen. When the metabolic nitrogen estimated from the dry matter consumed was substituted for the determined value in the preceding calculation, the average biological value was 61.6 (S.E., 3.20). The true digestibility was 89.6 (S.E., 1.12) when determined values for metabolic nitrogen were used and 89.9 (S.E., 1.26) with the calculated value.

The authors chose to test these methods of estimation with a protein of higher biological value; i.e., whole egg. Hawley et al. ('48) reported a biological value of 94 and a true digestibility of 98 for powdered defatted whole egg proteins in 7 subjects. For the 10 subjects of group 2 of the present studies, the determined biological value was 90.0 (S.E., 2.23) and the value derived from estimated endogenous nitrogen was 91.8 (S. E., 2.39). When both metabolic and endogenous

TABLE 5

Determined and calculated biological values and true digestibilities of the protein in sunflower seed flour and in whole egg

	DETERMINED VALUES, THOMAS METHOD		CALCULATED VALUES		
	Biological value	True digestibility	Biological value [1]	Biological value [2]	True digestibility [3]
	%	%	%	%	%
Sunflower (14 subjects of groups 2 and 3)	60.4 ± 2.28	89.6 ± 1.12	61.8 ± 2.94	61.6 ± 3.20	89.9 ± 1.26
Egg (10 subjects of group 2)	90.9 ± 2.23	92.8 ± 1.39	91.8 ± 2.39	92.0 ± 2.35	94.9 ± 1.36

[1] Calculated by using creatinine N × 3.984 in place of determined endogenous nitrogen value.

[2] Calculated by substituting an endogenous value derived from creatinine and an estimated value for fecal metabolic nitrogen using 0.88 mg per gram dry matter consumed.

[3] Calculated by allowing 0.88 mg fecal nitrogen per gram dry matter consumed.

nitrogen were estimated, the biological value was 92.0 (S.E., 2.35). The determined and calculated digestibilities were 92.8 (S.E., 1.39) and 94.9 (S.E., 1.36), respectively.

Comparison of the derived and determined biological values cited above shows no statistically significant differences between them. This similarity of values does not indicate, however, that a general application of this method of estimation is justified. The values of 25.1 and 0.88 were tested with

a portion of the same population from which the values were derived, so that the agreement may well be greater than when checked with an entirely different group of subjects. The authors recommend further checking of the factors derived for the relationship between creatinine nitrogen and endogenous nitrogen, together with considerably more study of the correspondence between metabolic nitrogen and dry matter consumed, before general use of the factors would be recommended. The variability of these factors for men and older people should be checked before they are applied generally.

In instances when a value for endogenous nitrogen is estimated for use in determinations of protein minima and in biological value calculations, as was the case in the work of Hegsted et al. ('46), an endogenous value derived from urinary creatinine nitrogen might be preferable to one based on the factor of 2 mg per basal calorie proposed by Smuts ('35) and used by the above authors.

SUMMARY AND CONCLUSIONS

Data from studies during three consecutive years have been combined in a study of the endogenous metabolism of women 19 to 30 years of age. Twenty-five subjects participated in the entire study. In addition, biological values were determined for sunflower seed flour and for whole egg.

The results were as follows:

1. In 39 determinations of endogenous urinary nitrogen it was found that in the majority of cases relatively constant values occurred during the last 5 days of a 14-day low-nitrogen feeding period. Shorter periods would have been unsatisfactory in most instances.

2. The mean daily endogenous urinary nitrogen excretion was 1,454 $\pm$ 30.8 mg for determinations on 25 subjects. When these endogenous values were expressed in milligrams per kilogram of body weight, milligrams per square meter of sur-

face area and milligrams per basal calorie, the average values were 25.2 ± 0.65, 898.0 ± 17.0 and 1.14 ± 0.021, respectively.

3. The creatinine nitrogen constituted one-fourth of the total endogenous urinary nitrogen (actual mean value, 25.1% ± 0.40).

4. The coefficient of variation of the mean value for the $\frac{CN}{EN}$ ratio was 8.1%. For the endogenous nitrogen this coefficient was 10.6%, and it was 13.0, 9.5, and 9.4% for the endogenous data expressed per kilogram, per square meter, and per basal calorie, respectively.

5. For each gram of dry matter consumed, the average metabolic fecal nitrogen was 0.88 ± 0.028 mg.

6. When fed to 23 subjects, sunflower seed flour protein had a biological value of 61.2 ± 1.71 and was 89.9 ± 0.86% digestible. For 10 of these subjects, the protein of whole egg showed a biological value of 90.9 ± 2.23 and was 92.8 ± 1.39% digestible.

7. Endogenous urinary nitrogen, estimated from the creatinine nitrogen × 3.984, together with estimated metabolic fecal nitrogen (obtained by multiplying 0.88 by the grams of dry matter consumed), was used in estimating the biological values of sunflower seed flour and of whole egg; resulting values were 61.6 ± 3.20 and 92.0 ± 2.35, respectively.

For these subjects, expression of the endogenous nitrogen as a multiple of urinary creatinine reduced the variation between individual values more than when body weight, surface area or basal metabolism served as a basis of expression, as evidenced by the coefficients of variation for the mean values. The differences between the coefficients proved significant only in a comparison of the creatinine expression with that for body weight. On the basis of the data herein reported, the authors recommend use of values for endogenous nitrogen derived from creatinine nitrogen × 3.984 only in estimation of the biological values and nitrogen minima of adult female populations. It is planned to test this factor with other segments of the population.

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TRANSAMINATION REACTION IN NORMAL AND B_6-DEFICIENT HAMSTERS[1]

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TWO FIGURES

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The B_6 group of vitamins has been implicated in transamination reactions ever since Snell ('44) first suggested this function as a possibility. Since then evidence has accumulated pointing toward the probability that pyridoxal phosphate and pyridoxamine phosphate serve as the prosthetic groups of bacterial and animal transaminases (Lichstein, Gunsalus and Umbreit, '45; Umbreit, O'Kane and Gunsalus, '46; Kritsman and Samarina, '46; Schlenk and Fisher, '47; Ames, Sarma and Elvehjem, '47; and O'Kane and Gunsalus, '47). Schlenk and Snell ('45), and Ames, Sarma and Elvehjem ('47) found that the rate of transamination in the tissues of B_6-deficient rats amounted to only about 40 to 60% of that found in the tissues of control animals. It was recently reported that a clear-cut B_6 deficiency may be produced in hamsters. The deficiency results in arrest of growth, muscular weakness, progressive malnutrition, fur changes, loss of fat tissue, pronounced atrophy of lymphoid tissues, notably the thymus, and irregular but markedly increased excretion of xanthurenic acid (Shwartzman and Strauss, '49). For the purpose of further characterization it seemed of interest to carry out the following studies on the transamination reaction in the tissues of deficient hamsters.

[1] These studies were made possible by a grant from the Commonwealth Fund.

EXPERIMENTAL

Weanling Syrian hamsters weighing between 27 and 30 gm were used for the studies. The experimental diet 3 previously described (Shwartzman and Strauss, '49) was modified to increase the casein content to 25% and the thiamine concentration to 16 mg per 10 gm of food mixture. The previously used salt mixture 2, U.S.P. XII, was replaced by the salt mixture 4 of Phillips and Hart ('35).

The control animals were kept at first on the B_6-deficient diet. After a 13- to 16-day period of depletion they were given daily subcutaneous injections of 50 μg of pyridoxine. Some control animals were allowed feed ad libitum, while others were pair-fed; i.e., the food intake was restricted to 2 to 2.5 gm per day, which was a near-starvation diet.

A few experiments were conducted on rats which were placed on a diet consisting of 18% casein, 75% sucrose, 3% dehydrogenated vegetable oil and 4% salt mixture (salt mixture 2, U.S.P. XII), supplemented with the vitamin solutions used for the hamsters but with the thiamine intake reduced to 10% mg. The pair-fed control animals were given daily injections of 50 μg of pyridoxine.

The animals were stunned and exsanguinated; the heart was removed and placed on ice; it was cleaned of extraneous material, sliced, rinsed in ice water, blotted on filter paper, weighed and homogenized in a Potter-type glass homogenizer containing 0.1 M phosphate buffer. One milliliter of homogenate represented about 20 to 25 mg of wet tissue. The homogenate was dialyzed for about three hours in a cellophane bag which was agitated in an ice cold bath containing the buffer. The dialysis apparently removed some metabolically active material. Normally not more than 20 minutes elapsed between the killing of the animals and the beginning of the dialysis period. In a few experiments, however, several animals were killed simultaneously and their hearts stored at about —30°C. The material was usually worked up within two to 5 days, although storage up to 19 days did not seem to have any deleterious effect on

transaminase activity. Studies were made on the α-keto-glutaric acid-aspartic acid system. The amount of oxalacetic acid formed was determined by measuring the amount of CO_2 liberated from it on the addition of aniline citrate (Ames and Elvehjem, '46). The results were corrected for "basal activity" and for "zero-tissue" concentration. Preliminary tests had satisfied us that there was no loss of oxalacetic acid during the 10-minute experimental period. Any oxalacetic acid formed by other than the transamination reaction was likely to be corrected for in the "basal activity," which was determined in a reaction mixture which contained all the experimental ingredients except α-keto-glutarate. The transamination rate was expressed in terms of microliters of CO_2 liberated per milligram of dry weight tissue nitrogen.

Hamsters are sensitive to changes in external temperature, so that transamination values obtained during warm weather were inordinately high in the normal as well as in the deficient animals. For this reason, each series of experiments had to be analyzed separately and no over-all averages were computed. Table 1 gives the average values for a typical series which consisted of 4 pair-fed and 6 deficient hamsters.

The transamination rate in the deficient group was about 30 to 40% lower than that of the control group; this difference was significant below the 2% level ($P = 0.–15$). Table 2 summarizes the results of 4 additional series of experiments, one of which was conducted on kidney homogenates.

The activity curves obtained for ad libitum-fed and for pair-fed animals were almost superimposable; age and food intake could, therefore, be eliminated as causes for the lowered activities of the deficient groups (fig. 1).

In deficient rats the rate of transamination was reduced to 50% of that of the control group ($P = 0.0002$), which compares with the 40 to 60% activities reported in the literature by Schlenk and Snell ('45), and Ames, Sarma and Elvehjem ('47; see fig. 2).

In the normal hamster the rate of transamination increased with age. There was a tendency for the transamination rate

to decrease as the duration of the deficiency period was prolonged. This finding, however, was subject to considerable individual variation.

The rate of transamination was found to be quite sensitive to changes in pH and was reduced to practically zero at a pH of 5.

TABLE 1

Transamination rates in normal and pyridoxine-deficient hamster hearts[1]

	AVERAGE OF 4 HAMSTERS	AVERAGE OF 6 HAMSTERS	% ACTIVITY OF DEFICIENT GROUP AS COMPARED WITH CONTROL GROUP
Diet	pair-fed	deficient	
Initial weight (gm)	31.7	25.6	
Final weight (gm)	27.0	25.8	
Age in weeks	6½	6	
Basal activity:			
Tissue level			
0.00 mg N	45.0 µl	63.5 µl	
0.05 mg N	1.8 µl	1.4 µl	78
0.10 mg N	3.8 µl	3.4 µl	89
0.20 mg N	7.6 µl	6.9 µl	91
0.30 mg N	11.4 µl	10.4 µl	91
Transamination rate, corrected:			
Tissue level			
0.00 mg N	61.0 µl	79.7 µl	
0.05 mg N	20.8 µl	11.0 µl	53
0.10 mg N	42.1 µl	21.9 µl	52
0.20 mg N	67.4 µl	39.3 µl	57
0.30 mg N	84.0 µl	49.6 µl	59

[1] Warburg apparatus, main compartment: 0.5 ml 0.25 M mixed phosphate buffer, pH 7.5, 0.3 ml 0.2 M aspartate, homogenate and 0.1 M buffer to a total volume of 2.0 ml; one side arm: 0.5 ml 0.1 M α-keto-glutarate; other side arm: 0.5 ml aniline citrate. Temperature, 37.1 to 37.4°C. Gas phase = air.

Tissue nitrogen determined by micro-Kjeldahl method.

The *in vitro* addition of pyridoxal at levels ranging from 5 µg to 20 mg failed to produce any significant effect on the tissues of deficient as well as control animals, even when 1 to 1.5 mg adenosine triphosphate (ATP) were added simultaneously. This compares with the findings in rats by Ames, Sarma and Elvehjem ('47; see table 3).

The *in vitro* addition of pyridoxal phosphate [2] at levels ranging from 1 μg to 2 mg failed to raise the rate of transamination of deficient hamster tissues. Ames, Sarma and

TABLE 2

Summary of transamination experiments in 4 additional hamster series

	July, 1949	Nov., 1949	March, 1950	July, 1950
Tissue	kidney	heart	heart	heart
Controls:				
Number of animals	4	2	5	2
Initial weight (gm)	25.8	32.3	25.9	28.8
Final weight (gm)	77.0	27.3	30.3	29.0
Age in weeks	9½	7½	8½	7½
Experimental period in weeks	7½	5½	4½	3½
Diet	mixed vegetables ad lib.	casein ad lib.	casein pair-fed	casein pair-fed
Deficient:				
Number of animals	2	2	5	7
Initial weight (gm)	27.0	31.5	26.4	26.9
Final weight (gm)	27.5	36.3	22.2	24.4
Age in weeks	9½	11½	7½	6½
Experimental period in weeks	7½	9½	3	2½
Transamination:				
Tissue level in mg N	0.5	0.5	0.3	0.3
Normal rate in μl CO_2	36.9	90.5	44.5	62.6
Deficient rate in μl CO_2	23.2	51.0	30.7	45.8
% activity of deficient group as compared to normal group	63	56	69	73
Standard deviation of normal group	4.17	1.41	5.44	0.17
Standard deviation of deficient group	4.24	7.00	6.99	4.66
Probability	0.035	0.030	0.015	0.005

Elvehjem ('47) reported a rise in activity produced in their deficient rats by 50 μg of pyridoxal phosphate (table 3).

Some deficient hamsters were given an initial booster dose of 50 μg of pyridoxine and subsequent daily injections of 10 μg. They began to gain weight immediately but transami-

[2] We are indebted to Dr. W. W. Umbreit of Merck Research Laboratories for this material.

nase activity was not fully restored until the treatment had been continued for about three weeks.

Control ad libitum-fed and pair-fed hamsters were given subcutaneous injections of desoxypyridoxine [3] in doses of

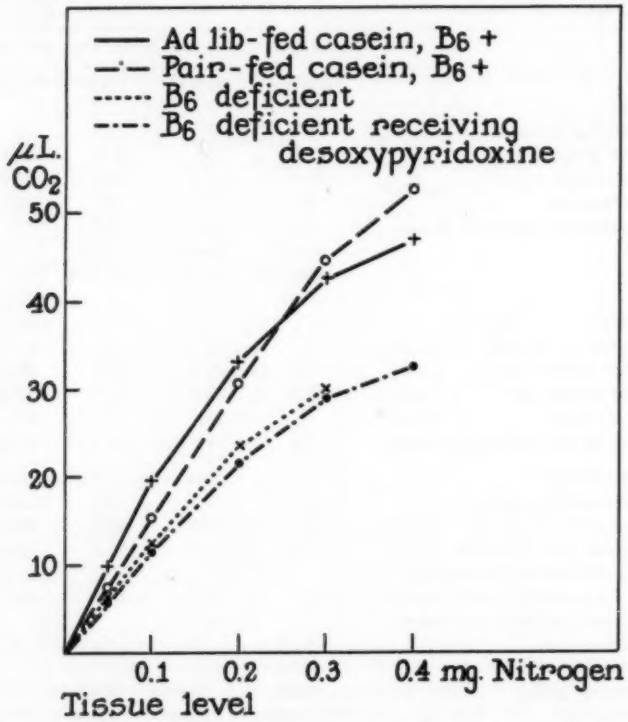

Fig. 1 The effect of diet on the transamination rate in hamster heart.

50 μg three times a week. Their weights continued to parallel those of the uninjected ad libitum-fed controls and their transamination rate fell within the normal range.

When pyridoxine-deficient hamsters were given similar injections of desoxypyridoxine, they lost weight more rapidly

[3] Obtained through the courtesy of Merck and Co.

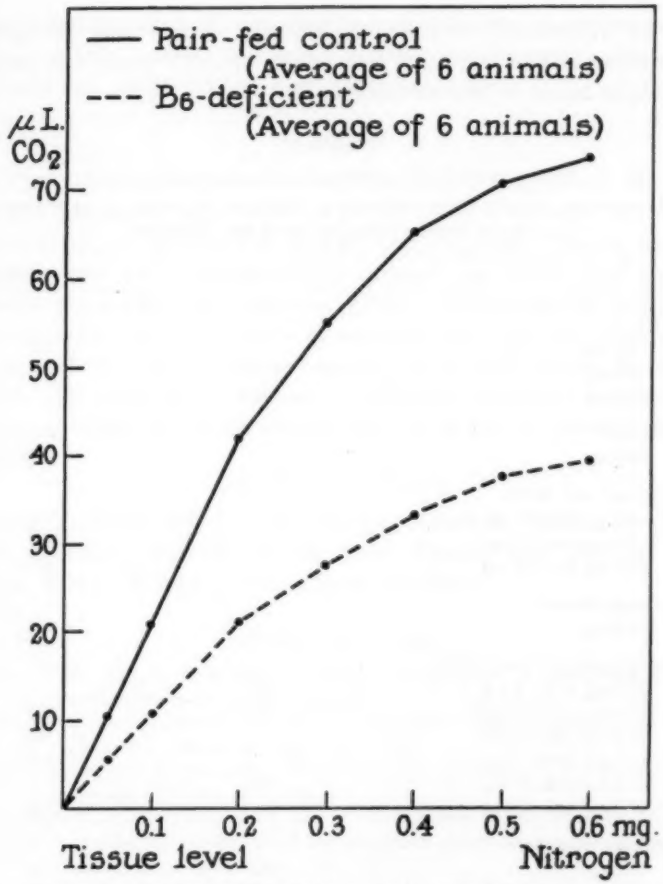

Fig. 2 Transamination rates in normal and B_6-deficient rats.

than did their deficient mates and appeared to be generally in worse condition. However, the transamination rate was found to be only slightly lower than that of the deficient group and this difference proved to be of no statistical significance (fig. 1).

The *in vitro* addition of desoxypyridoxine at levels ranging from 10 to 150 mg did not lower the transaminase ac-

tivity of control or deficient animals. Forty and 80 mg of the drug reinforced with 1 to 50 mg of ATP tended to cause a slight boost in activity (table 3).

TABLE 3

The effect of in vitro additions of pyridoxine derivatives

(Transaminase activity in the presence of additions expressed as percentage of activity found in the absence of such additions)

	B_6+ CONTROL	B_6− DEFICIENT
	%	%
ATP:		
1.2 mg	...	109
5.0 mg	112	103
10.0 mg	119	...
Pyridoxal:		
20.0 mg	...	114
Pyridoxal and ATP:		
50 μg and 1.2 mg	...	112
2.0 mg and 1.2 mg	...	107
20.0 mg and 1.2 mg	...	127
Desoxypyridoxine:		
80.0 mg	...	106
Desoxypyridoxine and ATP:		
20.0 mg and 5 mg	122	...
40.0 mg and 1 mg	137	116
40.0 mg and 5 mg	...	154
80.0 mg and 5 mg	...	140
80.0 mg and 10 mg	187	...
80.0 mg and 50 mg	...	182
Pyridoxal phosphate:		
1.0 μg	105	92
2.5 μg	119	98
25.0 μg	102	110
100.0 μg	...	109
2.0 mg	...	121

Succinoxidase activities were studied in a few animals, following the method of Schneider and Potter ('43). Homogenation was carried out in distilled water. The tissues were not subjected to dialysis and were always used fresh. The

activities found in B_6-deficient hamster hearts compared with those of the control animals. Similar results were obtained in rats by Ames, Sarma and Elvehjem ('47).

SUMMARY

The transamination rate of the heart muscle of B_6-deficient hamsters was found to be 30 to 40% lower than that of control ad libitum-fed or pair-fed animals. The *in vitro* addition of pyridoxal alone, pyridoxal and ATP, and pyridoxal phosphate was without effect. Injections of desoxypyridoxine into experimental animals and the *in vitro* addition of the drug alone or together with ATP failed to produce any significant changes. Deficient hamsters exhibited succinoxidase activities comparable to those of control animals.

ACKNOWLEDGMENTS

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MILKING TECHNIQUES AND THE COMPOSITION OF GUINEA PIG MILK[1]

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TWO FIGURES

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Different species of animals have different nutritional needs and digestive abilities. This fact is well illustrated by the wide differences in the composition of the milk of different species (table 1). Each of these milks must be well adapted to the young of its own kind, but in many cases it cannot support growth in the young of another species.

It is believed that a comparative study of the milks of different species may offer clues to differences in nutritional requirements which might otherwise be overlooked. Such information would be of particular value in the case of those species which are commonly used for laboratory experiments in nutrition. This report presents data from an extensive study of the milk of the guinea pig.

EXPERIMENTAL

Milking machine design

Several investigators have described miniature milking machines (Cox and Mueller, '37; Temple and Kon, '37; Glimstedt, Moberg and Widmark, '37; Houston and Kon, '39; Mueller, '39; Kahler, '42). A simplified design which

[1] This study was supported in part by grants from the Robert Gould Foundation, Inc., Cincinnati, Ohio, and the Herman Frasch Foundation, New York City.

retains the desirable features recommended by previous workers is shown in figure 1. This apparatus permits adjustment of the degree of vacuum, the frequency of application, and the relative length of the suction and release periods in each cycle. A Gast Portable, Rotary Air Blast and Suction Apparatus [2] has been used for adjusting the level of vacuum, although a water pump equipped with a suitable vacuum reg-

TABLE 1

The composition of milk — The "most nearly perfect food"

	PORPOISE [1]	RAT [2]	REINDEER [1]	HUMAN [3]	COW [3]	GUINEA PIG
Water (%)	41.1	68.3	65.3	88	87	83.56
Solids (%)	58.9	31.7	34.7	12.4	12.8	16.44
Calories/100 gm	462	191	235	71	69	77
Fat (%)	45.8	14.8	19.7	3.8	3.8	3.92
Lactose (%)	1.31	2.8	2.6	7.0	4.8	3.02
Ash (%)	0.57	1.50	1.43	0.21	0.71	0.82
Protein (%)	11.2	11.8	11.9	1.2	3.3	8.10
Casein (%)		9.2	8.7	0.4	2.8	6.62
Whey protein (%)		1.5	2.2	0.6	0.6	1.48
Reichert Meissl number		4.9	34	0.8	28.8	0.41
Polenske number		11.7	1.1	1.2	1.5	1.47
Iodine number		41	23	61.6	38.4	79.5
Saponification number		231	236	204.7	248.0	196

[1] Values collected from many sources.

[2] Values from: The composition of milk from stock rats and an apparatus for milking small laboratory animals, W. M. Cox and A. J. Mueller, J. Nutrition, *13:* 249–261, 1937.

[3] Values from: The Composition of Milks, Icie G. Macy et al., Natl. Res. Council Bull. 119, 1950.

ulator may be substituted. The motor used to turn the stopcock on the suction flask is equipped with a rheostat to permit variations in the number of pulsations per minute. The stopcock is lubricated with a light vaseline to allow easy rotation. Catheter tubing is used as leads from the teat cups to the small Erlenmeyer flask employed to collect the milk. A variety of teat cups were made by "flaring" the ends of glass tubing varying from 4 to 6 mm in outside diameter.

[2] Arthur H. Thomas Co., Philadelphia.

The flared end shoud be large enough to give a vacuum-tight seal against the surface of the gland without permitting the gland to be sucked into the tubing. The tubing should be large enough to allow room for the expansion of the teat which occurs during milking.

Milking techniques

It has been observed that the level of vacuum (10 to 11 inches of mercury) and the pulsations (25 per minute) for satisfactory milking of guinea pigs agree with the recommendations (20 to 30 cm of mercury and 20 to 30 pulsations per

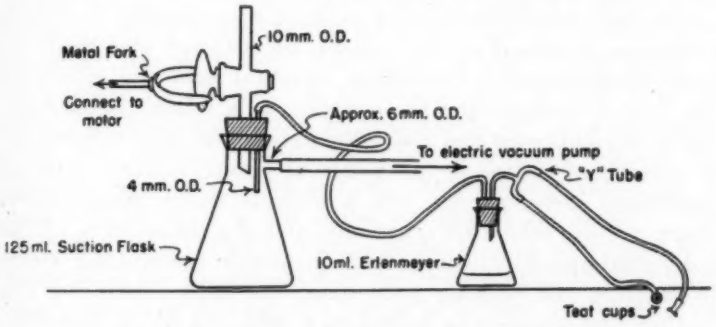

Fig. 1 Diagram of milking apparatus for guinea pigs.

minute) of Glimstedt et al. ('37). Any appreciably greater vacuum or more rapid pulsations may cause discomfort to the animal and a cessation of milk flow.

In the first milking tests, account was taken of the observation by Glimstedt et al. ('37) that the female ceases to lactate when the young are removed from the cage for periods longer than 24 hours. The young were removed at 9 A.M. and the mother was milked at 4 P.M. the same day and at 9 A.M. the following day. The young were returned to the cage for a period of 24 hours and the procedure repeated. In this manner regular milking intervals were established and are referred to as "alternate milkings."

Later, in order to obtain all the milk from the female guinea pig and at the same time maintain a high degree of lactation, the following method was employed: The cage ($7'' \times 17'' \times 9\frac{1}{2}''$) was divided by a wire mesh partition through which the pig could see and hear her young. The young were separated permanently from the mother by placing them on the opposite side of the partition immediately after parturition. If the young were born in the morning, the first milking was at 4 in the afternoon of the same day, but if born in the afternoon the first milking was at 9 in the morning of the next day. This method proved very successful and is referred to as "daily milking."

Constant conditions during milking are essential to obtain maximum yields. For this reason an attempt was made to keep environmental conditions constant and the effect of the time of milking and the operator at a minimum. Irregularity in yields over and above the anticipated production may be attributed to loss or morbidity of young, adverse milking conditions including too low a milking room temperature, sharp noises, other interruptions, and the disposition of the individual animal which made it unsuitable for milking. Prior to starting the milking procedure, the cage containing the mother and young was moved to a table on which the milking machine was assembled. The motor and vacuum pump were turned on and after a few minutes the female was gently removed and stroked for several minutes. The animal was slowly turned into a sitting position on the table, situated so her back would be supported against the operator's body. She could see her young in the cage. Concern of the female for her young was often shown while being milked. Before applying the teat cups the glands were massaged for one or two minutes. Both cups were attached at the same time. They were held between two fingers of each hand. The remaining fingers were available to massage the glands. The teat cups can be slightly pulled to stimulate flow of milk and to prevent congestion. This may be irritating, however, and should not be done if the animal objects. Pulling of the cup at the

close of milking was found to be advantageous in voiding the gland of the last of the milk. The massaging of the gland and the pulling of the cups was done in rhythm with the pulsations of the machine.

A small quantity of milk was usually obtained upon applying the teat cups. After two to 10 minutes a good flow of milk occurred, which in the dairy industry is referred to as the "letting down" of milk. The "let down" of milk in the guinea pig, especially in a good milker, is enhanced by constant conditions, so that the milk flows after the same interval and in the same manner at each milking. Milking was usually completed within several minutes. The flow of milk tends to stop suddenly, after which the pig becomes restless. Further milking after this time was impractical, as only a few drops of milk could be obtained. A continuous flow of milk was observed in some animals. The milk would flow in large quantities for several seconds and then would flow slowly but continuously for lengthy periods. Occasionally this type of milk flow changed to the usual pattern of the definite "let down." It was difficult to know when to stop milking of this latter type, for the animal may not become restless even after long periods of milking. This type of animal was milked the same length of time at each milking, usually 30 to 40 minutes.

Although some animals may not be easy to handle in the beginning, after a few periods of milking they usually respond without difficulty. Other animals can be milked readily at the start and during the process of "letting down" they are quiet, relaxed and sometimes emit noises.

Milk analyses and lactation performance

There were many individuals on the staff who were already engaged in making analyses of the kind desired. These staff members were asked to analyze a portion of the guinea pig milk. The results are shown in table 2. All of the analyses, except that for reduced ascorbic acid, were made on a composite sample of a number of guinea pig milkings. Reduced

ascorbic acid was measured on an aliquot taken immediately after each milking. Additional analyses were made on milk obtained by "alternate milkings." The results are shown in table 3. It will be noted that these values were obtained on

TABLE 2

The composition of a composite sample of guinea pig's milk

1.	Solids, %		16.44	
2.	Fat, %		3.92	
3.	Lactose, %		3.02	
4.	Ash, %		0.82	
5.	Protein, %		8.10	
6.	Casein, %		6.62	
7.	Whey protein, %		1.48	
8.	Non-protein nitrogen, %		0.19	
9.	Amino acids (mg/100 ml):			
	Arginine	800	Phenylalanine	607
	Histidine	380	Threonine	540
	Isoleucine	680	Tryptophan	280
	Leucine	1190	Basic tyrosine	720
	Lysine	1110	Acid tyrosine	600
	Methionine	300	Valine	940
10.	Fat characteristics:			
	Saponification number		196	
	Iodine number		79.5	
	Melting point °C.		25.2	
	Reichert Meissl number		0.41	
	Polenske number		1.47	
11.	Vitamins (values per 100 ml whole milk):			
	Vitamin A, μg		2	
	Carotenoids, μg		25	
	Riboflavin, μg (without hydrolysis)		405–423	
	Riboflavin, μg (with acid hydrolysis)		435	
	Thiamine, μg		50	
	Niacin, μg (without hydrolysis)		975	
	Niacin, μg (with acid hydrolysis)		1,110	
	Biotin, μg		4.5	
	Calcium pantothenate, μg		478	
	Folic acid total, μg		80	
	Folic acid free, μg		3	
	Reduced ascorbic acid, mg		28–54	
12.	Specific gravity 25°/25°		1.043	

TABLE 3

Daily milk and vitamin secretion in the lactating guinea pig

LACTATION DAY	FEMALE NUMBER	MILK YIELD			ASCORBIC ACID				RIBOFLAVIN [1]		NIACIN [1]		THIAMINE [1]	
		4 P.M.	9 A.M.	Total	4 P.M.	9 A.M.	Ave. 24-hr.	Total secreted	24-hr. sample	Total secreted	24-hr. sample	Total secreted	24-hr. sample	Total secreted
		gm	*gm*	*gm*	*mg/ml*	*mg/ml*	*mg/ml*	*mg*	*μg/ml*	*μg*	*μg/ml*	*μg*	*μg/ml*	*μg*
	730	3.20	6.57	9.77	0.663	0.590	0.62	6.0	8.50	83.0	2.37	23.2	0.29	2.8
1	720	3.63	7.07	10.70	0.513	0.553	0.53	5.8	5.47	58.5	2.07	22.1	0.25	2.7
	736	3.77	7.45	11.22	0.484	0.445	0.46	5.1	7.50	84.1	2.66	29.8	0.34	3.8
			Average	10.56			0.54	5.6		75.2		25.0		3.1
	730	7.89	5.19	13.08	0.228	0.304	0.26	3.4	3.64	47.6	13.80	180.5	0.44	5.8
3	720	11.42	7.29	18.71	0.273	0.298	0.28	5.3	3.47	64.9	13.65	255.4	0.70	13.1
	736	8.04	7.53	15.57	0.235	0.385	0.31	4.8	4.32	67.3	10.71	166.8	0.57	8.9
			Average	15.79			0.28	4.5		59.9		200.9		9.3
	730	4.10	2.96	7.06	0.405	0.468	0.43	3.0	3.72	26.3	17.52	123.7	0.57	4.0
5	720	3.44	4.18	7.62	0.395	0.468	0.43	3.3	4.59	35.0	18.15	138.3	0.57	4.0
	736	5.71	6.69	12.40	0.450	0.420	0.43	5.4	4.96	61.5	16.37	203.0	0.82	10.2
			Average	9.03			0.43	3.9		40.9		155.0		6.2
	730	1.41	4.08	5.49	0.300	0.263	0.28	1.5	6.59	36.2	14.60	80.2		
7	720	2.63	3.67	6.30	0.225	0.188	0.20	1.3	4.80	30.2	15.35	96.7	0.63	4.0
	736	4.00	4.92	8.92	0.305	0.455	0.38	3.5	5.41	48.3	19.65	175.3	0.88	7.8
			Average	6.90			0.29	2.1		38.2		117.4		5.9
	730	0.66	3.36	4.02	0.280	0.415	0.34	1.6	5.23	21.0	18.85	75.8	0.62	2.5
9	720	1.43	2.31	3.64	0.430	0.350	0.39	1.4	4.93	17.9	17.20	62.6		
	736	2.63	4.90	7.53	0.268	0.418	0.34	2.8	5.98	45.0	16.17	121.8	0.86	6.5
			Average	5.06			0.36	1.9		28.0		86.7		4.5

[1] Note: values are reported as micrograms per milliliter and total micrograms secreted in a 24-hour period.

the individual milkings and reflect the daily variation as well as total secretion of the various nutrients. All animals whose milk was used for chemical analyses were maintained on a stock ration consisting of oats, wheat and alfalfa hay supplemented with fresh green grass fed ad libitum daily. Typical lactation curves are presented in figure 2. The curve for "alternate milking" (curve A) showed a peak on the third

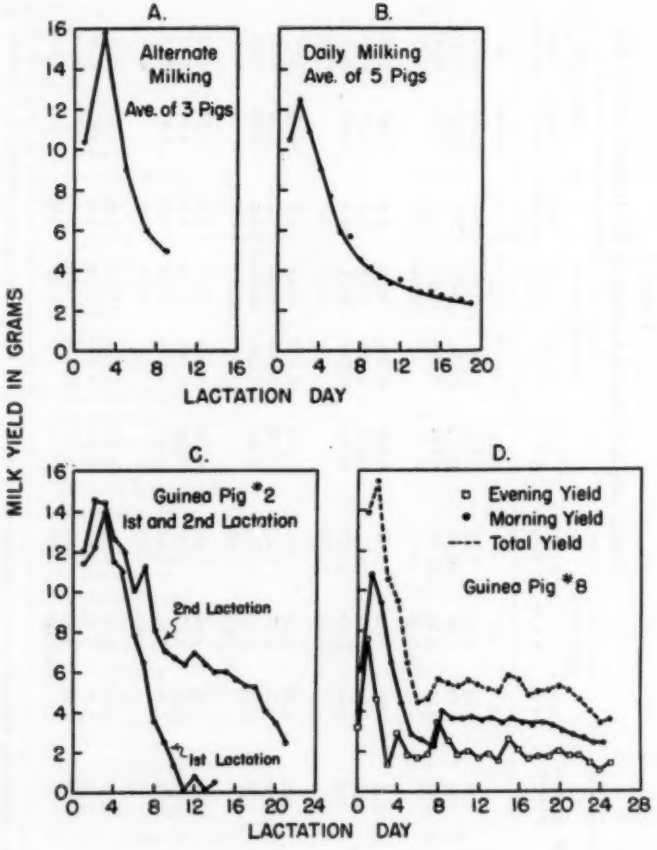

Fig. 2 Guinea pig lactation curves

day, or at the second milking of the animal. In most cases where the animal was milked daily (curves B, C, D) the peak occurred on the second day. The improved lactation performance of a guinea pig during her second lacation is shown in curve C. The relationship of evening and morning milk yield to the total daily yield is shown in curve D. These relationships are very similar to those reported for dairy cows.

DISCUSSION

There are some analyses of guinea pig milk in the literature (Neymark, '37; Abderhalden, 1899; Houston and Kon, '39; Engelbert, '45) which may be compared with the above. In general, there is good agreement except in the percentage of fat; others report fat contents of 5% or more. It should be recognized that there is considerable variation in the composition of the milk from different individuals of the same species, and from the same individual in different stages of lactation. This is well illustrated by the data reported in table 3. These data also point out the fallacy of attempting to show effects of diet on the composition of milk by reporting concentrations per 100 ml as the level of content of a nutrient in guinea pig milk when only an individual sample was taken. On this basis values for ascorbic acid range from 18.8 to 66.3 mg/100 ml, riboflavin from 364 to 850 μg/100 ml, for niacin from 207 to 1965 μg/100 ml and for thiamine from 25 to 88 μg/100 ml, and these variations are among individuals eating the same ration. Many analyses must be made to give a complete picture of the variations which may occur. The analyses in table 2 give no information regarding variation in composition but it is believed that they are based upon a most representative sample of guinea pig milk from our colony.

The lactation curves (fig. 2) and secretion data in table 3 indicate that the guinea pig can be used for measuring quantitatively the effect of diet on lactation performance. It is felt that the techniques described will permit a study of the various factors that may affect milk yield as well as nu-

trient composition. The procedure outlined above appears to obtain milk quantitatively from the lactating guinea pig in a manner similar to that usually employed for larger species such as the cow, goat or human. These techniques should provide a tool for studying lactation performance on a laboratory scale with savings of time, labor and expense.

SUMMARY

A milking machine of simplified design for use with small animals is described and the techniques used to obtain quantitative yields of milk during the entire lactation period of guinea pigs are discussed. Using these techniques, guinea pigs were milked for periods as long as 19 days without having nursed their young. A composite sample of guinea pig milk was analyzed for a larger number of constituents, and daily variations in milk yield, ascorbic acid, riboflavin and niacin concentrations are presented. Lactation curves for guinea pigs are presented which indicate that this animal is suitable for quantitative studies of the factors concerned with lactation.

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ALIMENTARY EXCRETION OF PHOSPHORUS^{32} IN RATS ON HIGH MOLYBDENUM AND COPPER DIETS [1]

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ONE FIGURE

(Received for publication February 17, 1951)

The point where phosphorus is excreted into the gastrointestinal tract is of particular interest in investigations of the nutritional interrelationships of other mineral elements with phosphorus. In these studies radioactive phosphorus^{32} provided a means of observing both the location of phosphorus excretion and the influence upon it of dietary differences.

Several investigators have demonstrated the excretion of P^{32} into the intestinal contents of rats after parenteral administration (Dols et al., '37; Cohn and Greenberg, '38). A disturbance of bone metabolism has been observed in cattle and rats fed high molybdenum diets and copper has been successfully used in the therapy of molybdenum toxicity (Comar et al., '49). In the present investigation a study was made of the influence of high molybdenum and high copper diets on the excretion of phosphorus into the contents of the various segments of the alimentary tract, as well as on the excretion of phosphorus in the feces and urine.

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PROCEDURE

Four groups of weanling piebald rats were fed a basal ration containing whole milk powder, sucrose, NaCl, ferrous sulfate, manganous sulfate, and thiamine hydrochloride. In addition, group 1 received 35 p.p.m. of Cu as copper sulfate and 80 p.p.m. of Mo as sodium molybdate mixed in the basal ration, group 2 received 35 p.p.m. of Cu, and group 3 received 80 p.p.m. of Mo. Group 4 received the basal diet only.

At approximately 150 days of age about 8 microcuries of carrier-free P^{32} [2] as H_3PO_4 in 1 ml of aqueous solution were administered intramuscularly in the thighs of the 6 rats of each group. At intervals of 0.5, 1, 6, 24, 96 and 168 hours after injection of the isotope, the rats were sacrificed and the tissues and contents of the stomach, duodenum, jejunum, ileum, cecum, colon, and rectum were isolated. The collected fecal and urinary excretions, the tissues, and organ contents were dry ashed with magnesium nitrate at 500°C. (Association of Official Agricultural Chemists, '45). The ash was dissolved in dilute nitric acid and made up to volume. Aliquots were used for the radioactive assays, using immersion type Geiger-Mueller tubes in conjunction with commercial scalers. The total phosphorus was determined by the colorimetric phosphomolybdate method (Fiske and Subbarow, '25).

RESULTS AND DISCUSSION

Alimentary tissues

Data were obtained for the per cent dose of P^{32} and per cent dose per milligram of total phosphorus in the segment tissues at the various intervals after administration of the isotope. At the 0.5 hour period, the total alimentary tissues contained approximately 2.5% of the P^{32} dose. The concentration increased to about 3% at the one to 6 hour periods and then decreased to approximately 1% by the 168 hour period. On the basis of per cent dose per milligram of total

[2] The P^{32} was received from the Oak Ridge National Laboratory after authorization by the Atomic Energy Commission, Isotopes Division.

phosphorus, all the alimentary segment tissues were essentially equivalent at approximately 0.3% at maximum concentration. No individual pattern was indicated among the 4 groups; all of them gave corresponding values that are probably within the range of normal biological variation.

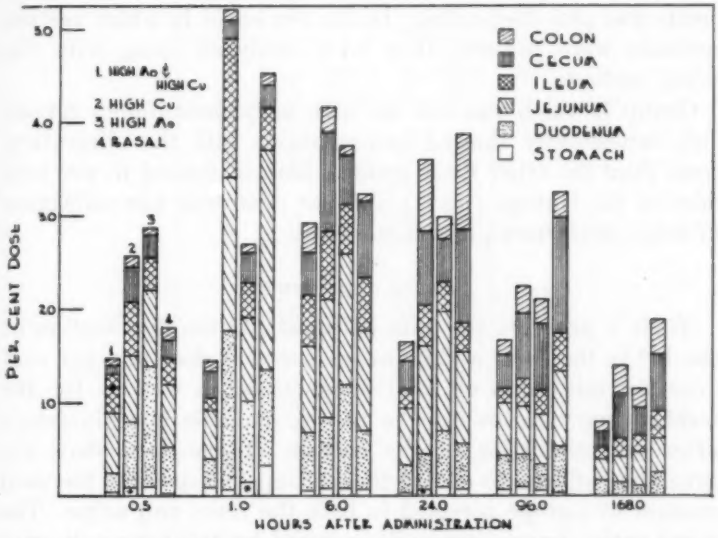

Fig. 1 Per cent dose of phosphorus[32] found in the contents of the various alimentary segments. *These samples were lost and the values reported are averages of the three corresponding values for the other groups.

Alimentary contents

The data presented graphically in figure 1 were obtained for the per cent dose in the contents of the various alimentary segments at intervals of from 0.5 to 168 hours after injection. These data show an appreciable concentration of the isotope in the contents of the alimentary tract, especially in the small intestinal segments and the cecum, by 0.5 hours after administration, a slight increase in one to 6 hours, followed by a decrease to approximately one-half of the maximum concentration by 168 hours, While the cecum had

considerable isotope present by the 0.5 to one-hour interval, the maximum concentration was reached at 6 hours and did not decrease appreciably until after 96 hours. In the case of the colon, the percentage of isotope was quite low during the first 6 hours but increased during the 24, 96, and 168 hour periods, when the amount of P^{32} in the upper intestinal segments was also decreasing. In the few cases in which rectum contents were present, they were analyzed along with the colon contents.

Group 1, which was fed the high molybdenum-high copper diet, consistently showed less excretion into the alimentary tract than the other three groups, both in regard to per cent dose of the isotope (fig. 1) and per cent dose per milligram of total phosphorus in the contents.

Feces and urine

Table 1 presents the data obtained for the distribution of the P^{32} in the feces and urine, as per cent dose and per cent dose per milligram of total phosphorus, as well as for the fecal: urinary ratios for the 24, 96, and 168 hour intervals after administration of the isotope. These data show appreciable differences among the 4 groups in regard to the total amount of isotope secreted in both the feces and urine. The basal ration group, group 4, excreted by 168 hours, through both the urinary and fecal pathways, a total of 33.74% of the P^{32} dose, while groups 3, 2, and 1 excreted 24.96, 20.64, and 15.05%, respectively.

The fecal: urinary ratio was 0.09, 0.01, 0.05, and 0.10 at 24 hours and 0.25, 0.28, 0.29, and 0.30 at 168 hours after administration of the isotope for groups 1, 2, 3, and 4, respectively, which indicates that both Cu and Mo decrease the amount of P^{32} excretion in both the feces and urine. Cohn and Greenberg ('38) reported that 48 hours after intraperitoneal injection of P^{32} in rats the fecal: urinary ratio was approximately 0.10, which checks quite closely with the above figures for our control rats and those for the animals that received both high molybdenum and high copper. The ratio

TABLE 1

Distribution of phosphorus[32] in the feces and urine

EXCREMENT	CONCENTRATION	GROUP 1			GROUP 2			GROUP 3			GROUP 4		
		24 [3]	96	168	24	96	168	24	96	168	24	96	168
Feces	[1]	0.45	1.28	3.05	0.04	2.13	4.54	0.33	2.32	5.66	0.83	3.17	7.74
Urine	[1]	5.17	7.80	12.00	7.03	6.54	16.10	6.47	8.00	19.30	8.58	9.67	26.00
Feces	[2]	0.06	0.20	0.10	0.01	0.09	0.15	0.07	0.14	0.12	0.13	0.02	0.11
Urine	[2]	0.41	0.28	0.22	0.80	0.29	0.28	0.86	0.71	0.35	0.70	0.33	0.25
Fecal: urinary ratio		0.09	0.16	0.25	0.01	0.33	0.28	0.05	0.29	0.29	0.10	0.33	0.30

[1] Per cent dose.

[2] Per cent dose per milligram of total phosphorus.

[3] Hours after administration.

obtained at 168 hours probably represents more closely what happens in the case of the rat after equilibrium of dietary minerals has been established.

SUMMARY

The excretion of intramuscularly administered P^{32} into the tissues and contents of the stomach, duodenum, ileum, jejunum, cecum, and colon of 4 groups of rats on rations containing Mo and Cu at levels of 80–35, 0–35, 80–0, and 0–0 p.p.m., respectively, was determined at intervals ranging from 0.5 to 168 hours after administration, as well as fecal and urinary excretion rates. The results were calculated as per cent dose and per cent dose per milligram of total phosphorus.

The group which was fed the high levels of both Mo and Cu excreted less of the isotope into the contents of the various alimentary segments than the other three groups, which were essentially equivalent with respect to excretion. Fecal and urinary excretion varied markedly among the 4 groups. The per cent P^{32} in the feces was 3.05, 4.54, 5.66 and 7.74, and in the urine 12.0, 16.0, 19.3, and 26.0 for the high Cu-high Mo, high Cu, high Mo, and basal ration rats, respectively, 168 hours after administration.

ACKNOWLEDGMENTS

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EDITORIAL REVIEW

THE BIOSYNTHESES OF TRYPTOPHAN AND NIACIN AND THEIR RELATIONSHIPS

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ONE FIGURE

(Received for publication March 10, 1951)

The observation by Krehl et al. ('45) that either the amino acid tryptophan or the vitamin niacin will support the growth of niacin-deficient rats initiated a period of vigorous research probing the nature of this phenomenon. This apparent relationship of tryptophan and niacin was of interest to workers in the fields of biochemistry and nutrition, since it appeared possible in this instance that tryptophan was serving as a precursor of niacin. If this were true, it would also imply that rats, though not dependent upon exogenous niacin, are capable of forming their own niacin only under certain nutritional circumstances.

Elucidation of the mechanism of the conversion of tryptophan to niacin has been carried out in large part with mutant strains of *Neurospora* characterized by their tryptophan or niacin requirements. That this relationship should be elucidated with such an organism rather than with animal tissue is due to the fact that several genetically different tryptophan- and niacin-dependent strains have been obtained during the past several years. These strains afford an opportunity to study individual reactions leading to tryptophan and niacin formation. First the present evidence concerning the biosynthesis of tryptophan and niacin in *Neurospora* will be reviewed, and then consideration will be given to the pertinent data relating to rats.

TRYPTOPHAN-NIACIN RELATIONS IN NEUROSPORA

Beadle et al. ('47) first noted that a mutant strain of *Neurospora* could grow when furnished niacin or tryptophan, and that this strain would also grow when furnished the tryptophan metabolite kynurenine. Shortly thereafter, Mitchell and Nyc ('48) found that 3-hydroxyanthranilic acid would support the growth of this strain, and advanced the suggestion that tryptophan serves as a precursor of niacin, and that the compounds kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid serve as intermediates in this conversion. This scheme, though logical in the light of the observations referred to above, presented a paradox which forced careful examination during the past two years of the relationship of these compounds in *Neurospora*. This paradox will be discussed shortly. Our present knowledge of the formation of tryptophan and its conversion to niacin is summarized in figure 1. Evidence for each of the indicated steps may be briefly summarized as follows:

a. Role of quinolinic acid

Following the observation that quinolinic acid is excreted by rats and that the amount excreted is increased by administering tryptophan or 3-hydroxyanthranilic acid (Henderson, '49a), the metabolism of quinolinic acid was studied in niacinless *Neurospora* mutants. Some of these niacinless strains were found to grow on high levels of quinolinic acid in place of niacin, but one, N-1, could not (Henderson, '49b; Bonner and Yanofsky, '49). Culture filtrates of this strain (N-1) were then tested for the possible accumulation of quinolinic acid and a large quantity was found (Henderson, '49b; Bonner and Yanofsky, '49). Similarly tested filtrates from other strains did not contain a significant amount (Bonner and Yanofsky, '49).

These observations led to the general hypothesis that the benzene ring of 3-hydroxyanthranilic acid is split in the 3–4 position to yield an unstable aliphatic intermediate of the

type shown in figure 1 (Henderson, '49a; Bonner and Yanofsky, '49). Two suggestions have been made concerning the relationship of this hypothetical intermediate to niacin and quinolinic acid. Henderson ('49b) has logically suggested that this compound undergoes ring closure, yielding quinolinic acid, which in turn is decarboxylated to yield niacin. However,

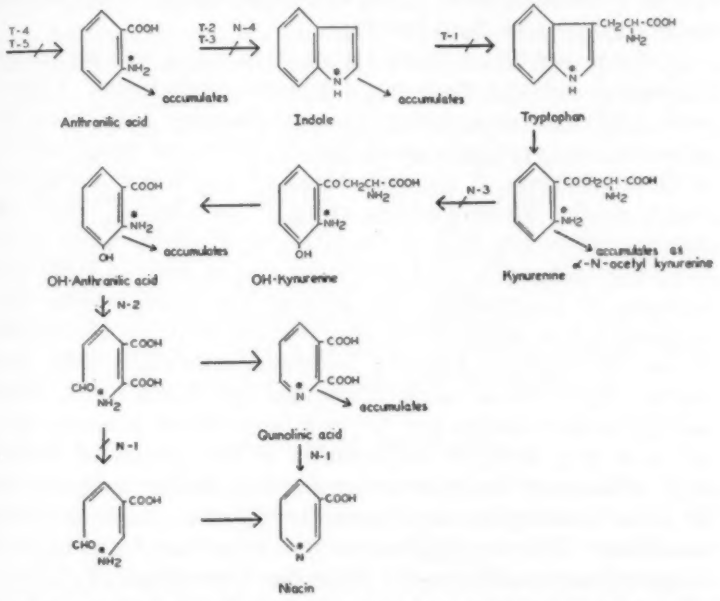

Fig. 1 Biosyntheses of tryptophan and niacin. Genetic blocks are indicated by strain numbers. N indicates niacinless strains; T indicates tryptophanless strains.

on a molar basis the growth-supporting properties of quinolinic acid are considerably less than those of either niacin or 3-hydroxyanthranilic acid, and it is difficult, therefore, to assign to quinolinic acid the role of a direct intermediate in niacin synthesis. To account for this low activity of quinolinic acid, it has been suggested (Bonner and Yanofsky, '49) that the aliphatic intermediate normally undergoes decarbox-

ylation prior to ring closure, and that niacin is the immediate product of ring closure. If, however, decarboxylation were prevented, the aliphatic intermediate might reasonably be expected to undergo ring closure and would give quinolinic acid as an alternative end product. The low biological activity of quinolinic acid would then result from either the reversal of this process followed by decarboxylation, or from direct decarboxylation in the 2 position.

At the present time there are no data which will critically distinguish between these two suggested mechanisms. Leifer et al. ('50), however, using tracer techniques, have recently advanced an hypothesis involving an additional complication in the mechanism of the conversion of 3-hydroxyanthranilic acid to niacin. These workers, using N^{15} as a tracer, observed that approximately 50% of the nitrogen of niacin comes from 3-hydroxyanthranilic acid, while the rest is derived from the nitrogen of the medium. To account for these results, the suggestion was made that a symmetrical diamino compound is the intermediate between 3-hydroxyanthranilic acid and niacin. More critical work (Partridge and Bonner, '51) with similar mutant strains and N^{15} as a tracer atom, however, has led to a very different explanation of the results of Leifer et al. This work has shown that mutants similar to that used by these investigators synthesize tryptophan under growing conditions. This tryptophan in turn gives rise to additional 3-hydroxyanthranilic acid. Thus the proportion of N^{15} recovered in niacin in such tracer experiments is primarily a function of the extent of tryptophan synthesis, and is not indicative of a particular mechanism for the conversion of tryptophan to niacin. A direct check of the conversion of 3-hydroxyanthranilic acid in resting mycelium has shown that the N^{15} content of the niacin formed is identical with that of the 3-hydroxyanthranilic acid used as substrate (Yanofsky and Bonner, '51). Thus, while the existence of an aliphatic compound such as that shown in figure 1 appears probable, it is still undetermined whether quinolinic acid is a direct intermediate in niacin synthesis in *Neurospora*.

b. Role of 3-hydroxyanthranilic acid

During preliminary observations of niacinless mutants of *Neurospora* (Bonner and Beadle, '46), it was noticed that the culture filtrates of one strain, N-2, possessed niacin activity for a second niacinless strain, N-4. These filtrates were fractionated and two active substances were isolated in crystalline form. One of these was found to be identical with synthetic 3-hydroxyanthranilic acid (Bonner, '48), which was also active for niacinless strain N-4 (Mitchell and Nyc, '48). On the basis of these observations — first, that 3-hydroxyanthranilic acid is accumulated by only one niacinless mutant strain, N-2, and, second, that this compound, although it does not replace niacin for N-2, can satisfy the niacin requirement of N-4 — 3-hydroxyanthranilic acid is believed to be a precursor of niacin in *Neurospora*. Resting mycelium experiments with mycelial pads of the various niacinless strains and of the parental strain have substantiated these observations (Yanofsky and Bonner, '51) and have rigorously proved that 3-hydroxyanthranilic acid is converted to niacin.

c. Role of kynurenine

As mentioned initially, kynurenine was suggested as a possible niacin precursor in *Neurospora* (Beadle et al., '47), since it was active in supporting the growth of strain N-4. Niacinless strain N-3 was found to be unable to use kynurenine for growth in place of niacin, while it could use 3-hydroxyanthranilic acid. Culture filtrates from this strain support the growth of N-4. The active substance present in such culture filtrates was isolated and identified as α-N-acetyl-kynurenine (Yanofsky and Bonner, '50). This compound is one seven-hundredth as active as niacin, and its accumulation is believed to result from the inability of N-3 to use kynurenine as a niacin precursor. Resting mycelium experiments have demonstrated the rapid conversion of kynurenine to niacin (Yanofsky and Bonner, '51). These data as a whole constitute

strong evidence in favor of the participation of kynurenine as a direct intermediate in niacin synthesis.

The compound 3-hydroxykynurenine has been postulated as the intermediate between kynurenine and 3-hydroxyanthranilic acid (Mitchell and Nyc, '48). This compound is known to occur naturally (Butenandt et al., '49), and has recently been tested and found highly active in replacing niacin for several niacinless *Neurospora* strains (Haskins and Mitchell, '49). To date no mutant has been reported which can use 3-hydroxyanthranilic acid and cannot use 3-hydroxykynurenine. Thus, while it appears likely that 3-hydroxykynurenine is an intermediate, rigorous proof of its participation is still lacking.

d. Tryptophan formation

Mutant strains requiring tryptophan for growth were among the earliest investigated. These strains, like the niacin-requiring strains, fall into distinct biochemical categories, and establish indole and anthranilic acid as tryptophan precursors. Some mutants which will grow on tryptophan but not on indole (Mitchell and Lein, '48) also accumulate indole and anthranilic acid in the culture medium. The conversion of indole to tryptophan is known to proceed by condensation of indole and serine (Tatum and Bonner, '44). Other strains will grow on either tryptophan or indole and accumulate anthranilic acid (Tatum et al., '43). A third class of mutant strains is known, and such strains are characterized by their ability to utilize either indole or anthranilic acid in place of tryptophan. How anthranilic acid is converted to indole is poorly understood. The amino group of anthranilic acid has been shown to form the nitrogen of the indole ring (Bonner and Partridge, '50; Partridge and Bonner, '51), and the available data suggest that the carboxyl of anthranilic acid is lost on indole formation (Nyc et al., '49). How anthranilic acid is formed is also poorly understood. Two compounds, shikimic acid and quinic acid, serve as apparent precursors of the aromatic ring in *Neurospora* (E. L. Tatum, personal

communication; Gordon et al., '50). However, they have not been established as true intermediates. Evidence for anthranilic acid formation from tryptophan through a "tryptophan cycle" has been recently presented (Haskins and Mitchell, '49). Whether such a cycle constitutes a major pathway of anthranilic acid synthesis appears questionable.

In general one can say, therefore, that tryptophan is formed from anthranilic acid through the intermediate indole. Tryptophan in part forms proteins, while a portion is metabolized to indolacetic acid and kynurenine. Kynurenine in turn is in part metabolized to such compounds as α-N-acetylkynurenine and possibly kynurenic and xanthurenic acids, but is also converted to 3-hydroxyanthranilic acid, which in turn is metabolized to niacin.

The scheme in figure 1 suggests that mutants of only two general types should be found, i.e., mutants requiring niacin or mutants requiring tryptophan. Strains responding to either tryptophan or niacin would not be expected. It was strains of just this type, however, which originally demonstrated that in *Neurospora*, as in other organisms, there is a relationship between tryptophan and niacin metabolism.

Experiments using N^{15} as a tracer have, therefore, been carried out to study the quantitative aspects of this relationship (Bonner and Wasserman, '50; Bonner and Partridge, '50; Partridge and Bonner, '51). It has been found that feeding either N^{15}-anthranilic acid or N^{15}-indole to various tryptophanless mutants and tryptophan-niacinless double mutants, leads to the same N^{15} content in the tryptophan and quinolinic acid isolated from the formed mycelium. These experiments have shown that *Neurospora* forms niacin from tryptophan and that this conversion constitutes the sole pathway of niacin formation.

In these experiments it was noticed that the tryptophan isolated had a lower N^{15} content than the compound fed. Furthermore, under limiting growth conditions this dilution with non-isotopic tryptophan was greatly increased. Since the mutants used in these investigations are presumably unable to

synthesize tryptophan from the components of *Neurospora* minimal medium, it is difficult to account for the presence of this non-isotopic tryptophan. An explanation of this observation has been offered in what we have termed the phenomenon of "leakage." Leakage can be defined as the ability of a mutant strain to form those compounds which it specifically requires for growth.

Leakage appears to be the most satisfactory explanation for the peculiar growth requirements of mutants which will grow when given either tryptophan or niacin (Partridge and Bonner, '51). Such strains, though apparently blocked in the synthesis of tryptophan, are capable of forming tryptophan when grown on niacin. At present we feel these strains do not contradict the scheme presented in figure 1, but represent strains in which there is considerable leakage past the genetic block. The leakage, however, is not sufficient to supply both the tryptophan and niacin required for growth, and thus the mutant must be furnished either more tryptophan or the tryptophan metabolite, niacin.

Thus all of the data obtained from studies with mutant strains of *Neurospora* are consistent with the view that tryptophan serves as a precursor of niacin, and that the pathway of niacin formation given in figure 1 is the sole such pathway in this organism.

TRYPTOPHAN-NIACIN RELATIONSHIPS IN RATS

The close relationship of tryptophan and niacin in rats need hardly be discussed here, since the interrelationship of these two compounds was first noted in animals and has been frequently reviewed (Krehl, '49). However, the evidence concerning the mechanism of the conversion of tryptophan to niacin will be considered briefly.

a. Quinolinic acid

Quinolinic acid has recently been isolated from the urine of various animals and identified as the compound which, upon

acid treatment, gives rise to a substance having niacin activity for *Lactobacillus arabinosus* (Henderson, '49a). The intraperitoneal injection of tryptophan or of 3-hydroxyanthranilic acid increases the amount of quinolinic acid excreted by rats (Henderson, '49a; Henderson and Hirsch, '49). Quinolinic acid relieves niacin deficiency in the rat (Henderson and Ramasarma, '49), but is far less effective than niacin or tryptophan (Henderson, '49b). Rat liver slices (Henderson and Ramasarma, '49; Schweigert and Marquette, '49; Krehl et al., '50), homogenates (Henderson and Ramasarma, '49; Schweigert and Marquette, '49) and enzyme preparations (Bokman and Schweigert, '50) convert 3-hydroxyanthranilic acid to quinolinic acid; however, neither tryptophan nor kynurenine gives rise to quinolinic acid when incubated with rat liver slices, nor will quinolinic acid give rise to niacin (Henderson and Ramasarma, '49) under these conditions. Thus quinolinic acid is closely related to tryptophan metabolism in the rat. Yet, as is the case with *Neurospora,* it cannot be stated at present in what manner quinolinic acid is involved in niacin formation.

b. 3-Hydroxyanthranilic acid

This acid has been found to replace niacin in the diets of rats (Mitchell et al., '48), but its activity is of the order of that of tryptophan rather than that of niacin. It has further been shown that addition of 3-hydroxyanthranilic acid to the diets of rats gives an increased urinary excretion of N^1-methyl nicotinamide (Albert et al., '48). Thus 3-hydroxyanthranilic acid appears to serve as an intermediate in niacin synthesis in rats, as it does in *Neurospora.* It has been recently shown that 3,4-dihydroxyanthranilic acid has growth-supporting activity roughly equivalent to that of 3-hydroxyanthranilic acid (Makino et al., '51). This compound is a logical oxidation product of 3-hydroxyanthranilic acid prior to cleavage of the benzene ring, and may represent an intermediate in niacin formation. Further work, however, is neces-

sary before the participation of 3,4-dihydroxyanthranilic acid in niacin formation is established.

c. Kynurenine

Kynurenine has been carefully tested for its ability to support the growth of rats maintained on a niacin-deficient diet, and found inactive (Krehl et al., '50). This finding is in agreement with an earlier report by Rosen et al. ('47) that kynurenine does not lead to increased urinary excretion of N^1-methylnicotinamide by rats. However, Kallio and Berg ('49) have reported that kynurenine does lead to a significant increase in the urinary output of the niacin metabolite N^1-methylnicotinamide. This report of Kallio and Berg is of particular importance, since kynurenine is biochemically labile (*cf.* the numerous papers of Y. Kotake); the negative results obtained in growth tests, therefore, could result from kynurenine destruction as well as from true inactivity. The presence of enzyme systems in the livers of several animal species which convert tryptophan to kynurenine through the intermediate formation of N-formylkynurenine has been demonstrated (Knox and Mehler, '50; Mehler and Knox, '50). This latter compound is inactive, however, in growth tests (Krehl et al., '50). No tissue preparation has been obtained which will convert kynurenine to quinolinic acid or niacin.

Isotope studies have provided indirect evidence for the participation of kynurenine in niacin synthesis in the rat. Heidelberger et al. ('49a, b) fed DL-tryptophan β-C^{14} to rabbits, rats and dogs. Kynurenine, kynurenic acid and N^1-methylnicotinamide were isolated from the urine and their C^{14} content determined. The position of the label was obtained by suitable degradation procedures. It was found that the isolated kynurenine contained C^{14} in the β-carbon atom, while the kynurenic acid contained C^{14} in the 3-position of the quinoline ring. The fact that the isolated N^1-methylnicotinamide did not contain any C^{14} shows that the pyridine ring of niacin is not formed from tryptophan by oxidation of the

benzene ring of kynurenic acid. When DL-tryptophan-3-C^{14} was employed, the tracer atom was found in the amide group of N^1-methylnicotinamide, suggesting that it is the benzene ring of tryptophan which becomes the pyridine ring of niacin.

Thus it has been shown that both kynurenine and niacin are formed from tryptophan. It has also been found that kynurenine does not satisfy the niacin requirement of rats in growth tests. Urinary excretion studies have indicated that kynurenine may be converted to niacin. It is difficult, therefore, to understand why kynurenine does not show some niacin activity in the rat. Experiments employing labelled kynurenine, with the subsequent isolation of niacin or N^1-methylnicotinamide, would go a long way toward clearing up this dilemma.

Thus the available data concerning the relationship of tryptophan and niacin in the rat present a certain paradox. Tracer experiments suggest that the scheme presented in figure 1 obtains in rats, yet growth experiments are not in entire agreement with such an interpretation. The answer to this dilemma is certainly not apparent, but in all probability rests in the complexity of the organism under investigation. For instance, critical proof that two compounds serve sequentially as precursors of the same end product is more difficult to obtain in investigations with rats than with microorganisms. This difficulty may in part be due to the fact that the activity of an exogenously supplied compound may differ from that of the same compound endogenously produced. Thus it appears difficult to establish valid criteria for judging the metabolic role played by a given compound in an organism whose various tissues are metabolically different.

An additional difficulty in assessing the data concerning tryptophan-niacin relationships in the rat is the fact that though rats are niacin independent if supplied tryptophan, formation of niacin is easily disturbed. Amino acids, low tryptophan proteins and corn grits are known to affect niacin formation adversely (Hankes et al., '48; Rosen and Perlzweig, '49). Tryptophan is known to be metabolized to various end

products, and the action of material such as corn grits suggests that the mode of tryptophan metabolism is strongly influenced by other substances present in the tissues. It should be further pointed out that while it has been possible in *Neurospora* to show that niacin formation occurs solely from tryptophan, there is no proof that the scheme of figure 1 obtains for all organisms. Mutant strains of *Aspergillus* are, in fact, known whose behavior suggests that the direct oxidation of anthranilic acid to 3-hydroxyanthranilic acid constitutes a pathway of niacin formation in this organism (Pontecorvo, '50).

In the face of these uncertainties, it can simply be stated that in general tryptophan appears to serve as a precursor of niacin in the rat essentially as shown in figure 1. Critical proof for each of the proposed steps, however, has not as yet been obtained.

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PROCEEDINGS OF THE FIFTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

THE HOTEL CLEVELAND AND THE PUBLIC AUDITORIUM, CLEVELAND, OHIO, APRIL 29–MAY 3, 1951

COUNCIL MEETINGS

Council Meetings were held in the Hotel Cleveland on Saturday, April 28, and Sunday, April 29. Formal actions of the Council are reported in the minutes of the business meetings.

SCIENTIFIC SESSIONS

The scientific program consisted of 7 half-day sessions at which 82 submitted papers were presented and a half-day session devoted to a symposium on the "Utilization of Protein." The symposium was held on the afternoon of May 1. Four papers were read by title.

BUSINESS SESSIONS

Two business meetings were held: one at 4:00 P.M., Monday, April 30, and one at 4:00 P.M., Wednesday, May 2. Items of business received attention as follows:

Monday, April 30, 4:00 P.M. The meeting was called to order by President Wendell H. Griffith. The President appointed Dr. W. A. Krehl and Dr. R. A. Gortner, Jr., to serve as a Tellers' Committee on the election of Officers. Ballots were transmitted to the Tellers' Committee by the Secretary.

The Council's recommendations for election to membership in the American Institute of Nutrition were submitted.

It was voted to approve the Council's recommendations. The following persons were elected to membership:

Conrad F. Asenjo
Francis H. Bird
Raymond Borchers
Wilma Denell Brewer
Wise Burroughs
George E. Cartwright
Cyril Lewis Comar
Esther Da Costa
Kate Daum
Robert John Evans
James Clarence Fritz
Claire E. Graham
Clark J. Gubler
Henry R. Kraybill
George V. Mann
Jean Mayer
Walter L. Nelson
Donald B. Parrish
Mary Louise Quaife
May S. Reynolds
Eldon E. Rice
Charlotte E. Roderuck
Clara Mae Taylor
George H. Wise

Requests from the Executive Committee for action on proposed changes in the by-laws of the Federation were submitted by President Griffith. A motion to approve the changes was carried. The changes were as follows:

By-Law 1. Change to read: "The Executive Comittee of the Federation shall consist of the President, the Secretary, and the immediate Past-President of each of the constituent Societies. In the absence of a member of the Executive Committee, his place may be filled by the Council of the appropriate Society."

By-Law 2. Change to read: "The Chairmanship of the Executive Committee shall be held in turn by the *Past*-Presidents of the constituent Societies, who shall succeed one another annually in the order of seniority of the Societies."

By-Law 8. Change to read: "The Executive Committee shall consider measures of advantage to the Federation as a whole. Any constituent Society may refer similar measures to the Executive Committee. The Executive Committee may take such action, or may authorize the Chairman or the Federation Secretary to take such action, as it may approve in accordance with the following voting procedure: A quorum shall be at least 14 members voting, including at least two representatives from each Society. Resolutions, to prevail, shall require the affirmative votes of at least two representatives from each constituent Society. The actions of the Executive Committee shall be reported to the Societies at the annual business meeting."

The report of the Treasurer, Dr. N. B. Guerrant, was submitted. A report of the Auditing Committee, consisting of Dr. Alex Black and Dr. R. W. Swift, was presented by Dr.

Swift. The Auditing Committee reported that in the Treasurer's books, receipts and disbursements are properly recorded and that his report as of April 10 is substantiated by the records.

The report of Dr. W. C. Russell, representative of the Institute of Nutrition to the Division of Biology and Agriculture, National Research Council, was submitted.

The Annual Report of the Editorial Board of the Journal of Nutrition was submitted by the Editor, Dr. George R. Cowgill. The report follows.

Annual Report of the Editor of the Journal of Nutrition

This annual report covers the volumes of The Journal of Nutrition published during 1950 as well as general matters arising since the last annual meeting of the American Institute of Nutrition in Atlantic City, New Jersey, April 20, 1950.

Papers published and related data

In 1949 we began the new policy of printing three volumes a year. Our present report therefore covers the second year under this policy. The volumes published in 1950 were numbered 40, 41 and 42. They contained 50, 50 and 51 articles, respectively, giving a total for the year of 151, compared with 127 for 1949. The average number per issue was 12.6, compared with 10.3 for the previous year. The number of pages per article proved to be 12.5, compared with 13.1 for 1949. The former plan of two volumes annually called for printing at least 1,500 pages per year; the present plan of three volumes per year means the printing of at least 1,800 pages of scientific matter. Actually, during the year 1950, subscribers received a total of 1,892 pages. During the past year 170 papers were submitted and 43, or 25.3%, were rejected. The corresponding figures for 1949 were 148 submitted and 31, or 21%, rejected.

Picture on front cover

In accordance with the policy voted by the editorial board at its 1949 meeting in Detroit, the first issue of each volume carried a new picture, a large one being used as a frontispiece accompanied by a short biography, and a smaller one on the front cover. The pictures were those of F. G. Hopkins, Graham Lusk and C. Eijkman, used in

this order. At its last meeting the editorial board voted to use the pictures of Magendie, Beaumont and Bernard for the year 1951.

Personnel of editorial board

At the last annual meeting three new members were elected to replace those whose terms expired. The new members were Vernon H. Cheldelin, Carl V. Moore and Harold H. Williams. In accordance with past policy, this group of names appeared last in the list published on the front cover of each issue.

Dr. D. M. Hegsted has been away since June, 1950, on an assignment in Peru. He offered to resign his board membership but was persuaded to consider himself as "away on leave," since it did not appear that he would be absent for the rest of his term as a board member.

Editorial problems

These continue to be about the same as those encountered in previous years. A meeting of the editorial board was held April 29, 1951, in the Hotel Cleveland, at which the various editorial problems were reviewed, an exchange of ideas obtained, the actions of the editor discussed, and other matters pertaining to the Journal and the operation of the editor's office considered. Many authors continue to submit manuscripts that have obviously not been prepared in accordance with the instructions printed on the inside back cover of every issue. The editor was instructed by the editorial board to feel free hereafter to return to the author for retyping any such papers if it was felt that this was desirable and would serve a useful purpose.

General comments

As editor I wish to express my appreciation of the services rendered by the individual members of the editorial board. All of them have been very cooperative. Frequently they have had to read more papers than usual during periods when many members were not available — summer months, for example. In return for this I have tried to give them fewer assignments later. They have been properly frank in stating their reactions to papers and they have tried to make their comments and criticisms as constructive as possible.

I should like also to express my appreciation of the work done by the staff of Wistar Institute, the cooperation and interest they have always displayed in solving printing problems as much as possible according to our wishes. There have been delays in appearance of issues due to factors beyond their control. They have never by their

attitude presented me with a problem. Their attitude has always been: How do you want this handled? How do you wish this to be printed? For all of this I am very grateful. If, now, we can somehow get the proper cooperation of every contributor, and thus reduce our handling of manuscripts to merely the slight rewriting and routine editorial work which every manuscript must have, we might feel that we had come closer to an editor's Utopia.

A motion was passed expressing appreciation to Dr. Cowgill for his outstanding work upon the Journal of Nutrition.

A progress report concerning the work of the Committee on Registry of Nutritional Pathology was presented by President Griffith.

The report of the Tellers' Committee on the results of the election of officers was received. Two hundrd and sixteen ballots were cast. The following officers were elected for the year beginning July 1, 1951:

President:
Clive M. McCray

Secretary:
James M. Orten

Vice-President:
Paul L. Day

Councillor:
Grace A. Goldsmith

Associate Editors:
Alex Black
Floyd S. Daft
Harry G. Day

The meeting was adjourned at 5:15 P.M.

Wednesday, May 2, 4:00 P.M. The meeting was called to order by President Griffith.

The report of the Tellers' Committee on the 15 members receiving the highest number of votes as suggestions for the Nominating Committee was received.

Dr. James M. Hundley gave a detailed report on his attendance at the Eighteenth International Physiological Congress in Copenhagen, August 15–18, 1950, as a representative of the American Institute of Nutrition.

President Griffith and Dr. L. A. Maynard spoke briefly upon the work that is being done with a view to the organization of an International Union of Nutritional Sciences.

A motion was passed to set the annual dues at $1.00 per year, as recommended by the Council.

Dr. E. S. Nasset discussed the work that is being done toward obtaining legislation permitting the use of unclaimed animals in city pounds for medical investigations. A motion was passed to approve a resolution from the Executive Committee of the Federation favoring such legislation, to be sent to the National Society for Medical Research and to legislators or organizations concerned.

Under "new business" a spirited discussion took place regarding a certification of specialists in human nutrition by the American Board of Nutrition.

The following motion was presented by Dr. Esther L. Batchelder: It is hereby resolved that the president be empowered to inform the members by mail upon a question involving the policy of the Institute, giving each an opportunity to express by mail (post card) his opinion on the matter. This will not be construed as a vote but as advice to the president in helping him carry out his responsibilities. The motion was adopted.

The meeting was adjourned at 6:00 P.M.

DINNER AND PRESENTATION OF AWARDS

The annual dinner of the Institute of Nutrition was held on Tuesday evening, May 1, in the Hotel Cleveland. The program consisted of the presentation of awards and the introduction of new members.

The Borden Award was presented to Dr. Paul György, University of Pennsylvania Medical School, for "his contributions to our knowledge of the nutritive importance of components of dairy products." The achievements of the medalist were described by Dr. W. J. Darby, Professor of Biochemistry, Vanderbilt University. Dr. Darby spoke of Dr. György's work upon calcium and phosphorus metabolism in rickets, riboflavin isolation and deficiency, biotin deficiency, and the interrelationship of casein, methionine and choline in

the development of fatty livers. Dr. György responded with a modest and interesting analysis of his work in nutrition.

The Osborne and Mendel Award was presented to Dr. Esmond E. Snell, Professor of Biochemistry, University of Wisconsin, for "contributions to the development and application of microbiological methods in nutritional and biochemical investigations." Dr. C. A. Elvehjem, Dean of the Graduate School, University of Wisconsin, reviewed the work of Dr. Snell. Dr. Elvehjem spoke of the importance of Dr. Snell's pioneer work in the development of bioassay methods of analysis and of his contributions to the science of nutrition. Dr. Snell responded humorously and delightfully with reminiscences concerning his work.

COMMITTEES FOR 1951–1952

President Wendell H. Griffith appointed the following committees for the year beginning July 1, 1951.

Nominating Committee

H. J. Deuel, Jr., Chairman
I. M. Hoobler
C. G. King
W. J. Darby
A. G. Hogan

Committee on Registry of Nutritional Pathology

H. Pollack, Chairman
W. H. Sebrell, Jr.
E. L. Sevringhaus
O. A. Bessey

Representatives on Joint Committee on Nomenclature

C. A. Elvehjem
E. M. Nelson

Representative to the Division of Biology and Agriculture and to the Food and Nutrition Board, National Research Council

W. C. Russell

Respectfully submitted,
JOSEPH H. ROE, *Secretary*
American Institute of Nutrition

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